

10 December 2010 | \$10

Science

 AAAS

EDITORIAL

- 1455** Cultivating Young Academies
Volker ter Meulen and Günter Stock

NEWS OF THE WEEK

- 1460** Australia to Test 'Mosquito Vaccine' Against Human Disease
- 1461** Shanghai Students Lead Global Results on PISA
- 1462** Italian Parliament Passes Controversial University Reforms
- 1462** A Government Niche for Translational Medicine and Drug Development
- 1463** From the *Science* Policy Blog
- 1464** Chinese Initiative Aims to Comprehend and Combat a Slimy Foe
- 1465** El Niño Lends More Confidence to Strong Global Warming
>> Report p. 1523
- 1466** The Beginning of the End for Africa's Devastating Meningitis Outbreaks?
- 1467** From *Science's* Online Daily News Site

NEWS FOCUS

- 1468** To Fight Illegal Fishing, Forensic DNA Gets Local
>> Science Podcast
- 1470** What Shall We Do With the X-ray Laser?
- 1472** American Schools of Oriental Research Annual Meeting
A Change of Biblical Proportions Strikes
Mideast Archaeology
Tracking the Med's Stone Age Sailors
Keeping Watch as the Old Kingdom Crumbled
- 1474** Chesapeake Crabs: Engineering a Rebound
- 1475** Will Homebody Researchers Turn Japan Into a Scientific Backwater?

LETTERS

- 1476** Travel Trade-Offs for Scientists
I. C. Burke
- Readers' Poll: Travel Trade-Offs for Scientists

- Cataloguing Soil Carbon Stocks
D. Gianelle et al.
- Credit to Plowright for Rinderpest Eradication
D. Robertshaw
- Optimizing Scientific Reasoning
J. R. Skoyles

1477 CORRECTIONS AND CLARIFICATIONS

BOOKS ET AL.

- 1478** Science Books for Fun and Learning—Some Good 2010 Choices
- 1482** Two from China
J. Liu and S. Li
- 1482** Browsers

POLICY FORUM

- 1483** Stagnant Health Technologies in Africa
K. Simiyu et al.

PERSPECTIVES

- 1485** Building a Better Battery
Y.-M. Chiang
>> Report p. 1515
- 1486** Genome Evolution in Plant Pathogens
P. N. Dodds
>> Reports pp. 1540, 1543, 1546, and 1549
- 1487** Sex and Sacrifice
R. H. Kessin
>> Report p. 1533
- 1488** First-Class Control of HIV-1
A. J. McMichael and E. Y. Jones
>> Report p. 1551
- 1490** Earth's Second Wind
L. R. Kump
- 1491** Gett'N-WASP Stripes
M. Gautel and E. Ehler
>> Report p. 1536
- 1493** Retrospective: Veronica Rodrigues (1953–2010)
K. VijayRaghavan and M. Bate

CONTENTS continued >>



page 1468



page 1478



COVER

An *Arabidopsis thaliana* seedling with downy mildew disease, caused by the oomycete pathogen *Hyaloperonospora arabidopsidis*. Four Reports beginning on page 1540 present comparative genomic analyses of several plant pathogens that shed light on their biology, including their virulence, dependence upon their host, and host specificity. Included are pathogens responsible for economically devastating diseases in crop plants. Also see the related Perspective on page 1486.

Photo: Ryan Anderson and John McDowell, Virginia Polytechnic Institute and State University

DEPARTMENTS

- 1451** This Week in *Science*
- 1456** Editors' Choice
- 1458** *Science* Staff
- 1459** Random Samples
- 1558** New Products
- 1559** *Science* Careers

REVIEW

- 1496** Scenarios for Global Biodiversity in the 21st Century
H. M. Pereira et al.
>> *Research Article p. 1503*

BREVIEW

- 1502** Slow Earthquakes Linked Along Dip in the Nankai Subduction Zone
H. Hirose et al.
Three types of temporally linked slow earthquakes may limit nearby buildup of stress.

RESEARCH ARTICLE

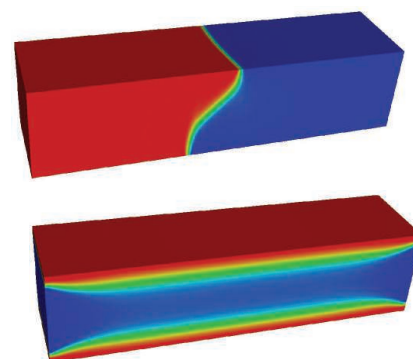
- 1503** The Impact of Conservation on the Status of the World's Vertebrates
M. Hoffmann et al.
Though the threat of extinction is increasing, overall declines would have been worse in the absence of conservation.
>> *Review p. 1496*

REPORTS

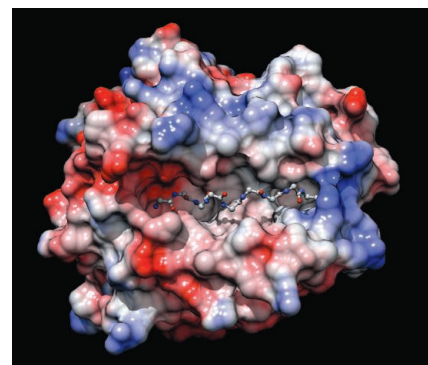
- 1510** Toroidal Dipolar Response in a Metamaterial
T. Kaelberer et al.
A material that embeds metal wire loops in a dielectric has properties consistent with an exotic electromagnetic excitation.
- 1512** Evidence of Supersolidity in Rotating Solid Helium
H. Choi et al.
Measurements on rotating frozen helium support the formation of a quantum, or supersolid, phase.
- 1515** In Situ Observation of the Electrochemical Lithiation of a Single SnO₂ Nanowire Electrode
J. Y. Huang et al.
Transmission electron microscopy reveals dimensional changes in a tin oxide nanowire as it intercalates lithium.
>> *Perspective p. 1485*
- 1520** Optomechanically Induced Transparency
S. Weis et al.
Radiation pressure is used to manipulate the optical properties of an optomechanical system.
- 1523** A Determination of the Cloud Feedback from Climate Variations over the Past Decade
A. E. Dessler
The climate-cloud feedback is positive, supporting current ideas about how atmospheric carbon dioxide affects global temperature.
>> *News story p. 1465; Science Podcast*

- 1527** Stochastic Late Accretion to Earth, the Moon, and Mars
W. F. Bottke et al.
Random impacts led to the late delivery of highly siderophile elements (noble metals) during the growth of these bodies.
- 1530** Thought for Food: Imagined Consumption Reduces Actual Consumption
C. K. Morewedge et al.
Imagining eating a food caused subsequent actual consumption of that food to decline.
>> *Science Podcast*
- 1533** Sex Determination in the Social Amoeba *Dictyostelium discoideum*
G. Bloomfield et al.
The three mating types of a model eukaryote are regulated by two small soluble proteins.
>> *Perspective p. 1487*
- 1536** Nebulin and N-WASP Cooperate to Cause IGF-1-Induced Sarcomeric Actin Filament Formation
K. Takano et al.
An alternative signaling mechanism for nucleating unbranched actin filaments is required for skeletal muscle maturation.
>> *Perspective p. 1491*
- 1540** Genome Evolution Following Host Jumps in the Irish Potato Famine Pathogen Lineage
S. Raffaele et al.
- 1543** Genome Expansion and Gene Loss in Powdery Mildew Fungi Reveal Tradeoffs in Extreme Parasitism
P. D. Spanu et al.
- 1546** Pathogenicity Determinants in Smut Fungi Revealed by Genome Comparison
J. Schirawski et al.
- 1549** Signatures of Adaptation to Obligate Biotrophy in the *Hyaloperonospora arabidopsidis* Genome
L. Baxter et al.
A group of papers analyzes pathogen genomes to find the roots of virulence, opportunism, and life-style determinants.
>> *Perspective p. 1486*
- 1551** The Major Genetic Determinants of HIV-1 Control Affect HLA Class I Peptide Presentation
The International HIV Controllers Study
MHC class I alleles are the major genetic determinants associated with people able to control HIV infection without therapy.
>> *Perspective p. 1488*

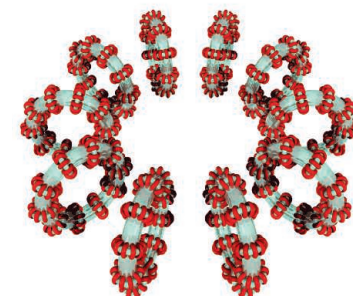
CONTENTS continued >>



pages 1485 & 1515



pages 1488 & 1551



page 1510

SCIENCEONLINE

SCIENCEEXPRESS

www.sciencexpres.org

Direct Conversion of *C. elegans* Germ Cells into Specific Neuron Types

B. Tursun et al.

Removal of a chromatin factor allows transcription factors to reprogram germ cells into neurons.

10.1126/science.1199082

Rotational Movement of the Formin mDia1 Along the Double Helical Strand of an Actin Filament

H. Mizuno et al.

Visualization of formin protein rotating along an actin filament gives insight into how it promotes actin assembly.

10.1126/science.1197692

Decreased Clearance of CNS β -Amyloid in Alzheimer's Disease

K. G. Mawuenyega et al.

Alzheimer's disease is associated with reduced β -amyloid clearance from the brain.

10.1126/science.1197623

Universal Quantum Viscosity in a Unitary Fermi Gas

C. Cao et al.

Viscosity studies of an ultracold gas of ^6Li atoms in two temperature regimes enable comparison with a string theory limit.

10.1126/science.1195219

Tunable Field-Control over the Binding Energy of Single Dopants by a Charged Vacancy in GaAs

D. H. Lee and J. A. Gupta

The electrostatic field of manganese atoms in gallium arsenide depends on its distance from a nearby arsenic vacancy site.

10.1126/science.1197434

SCIENCENOW

www.sciencenow.org

Highlights From Our Daily News Coverage

How Swine Flu Killed the Healthy

New research suggests that patients' own immune systems could have turned against them.

Killer Whales Mimic Friends and Foes

Orcas that do not share the same dialect can still copy one another's calls.

Did Climate Change Drive Prehistoric Culture Change?

Early Americans may have dramatically altered their life-styles in response to new weather patterns.

SCIENCE SIGNALING

www.sciencesignaling.org

The Signal Transduction Knowledge Environment

EDITORIAL GUIDE: Focus Issue—

Metabolic Signals

W. Wong

Complementing the 3 December *Science* special issue, this issue highlights signaling pathways with roles in metabolism.

RESEARCH ARTICLE: A Kinase-Independent Role for Unoccupied Insulin and IGF-1 Receptors in the Control of Apoptosis

J. Boucher et al.

PERSPECTIVE: Dependence Receptors—The Trophic Theory Revisited

P. Mehlen

Cells lacking both insulin and IGF-1 receptors are resistant to apoptosis.

RESEARCH ARTICLE: Regulation of the 26S Proteasome Complex During Oxidative Stress

X. Wang et al.

PODCAST

L. Huang and A. M. VanHook

Oxidative stress triggers release of the core catalytic subunit of the yeast 26S proteasome, enabling degradation of toxic oxidized proteins.

PERSPECTIVE: Lipid Signaling and Homeostasis—PA Is Better than PA-H, But What About Those PIPs?

N. T. Ktistakis

Phosphatidic acid links changes in pH triggered by glucose metabolism to phospholipid biogenesis in yeast.

RESEARCH RESOURCE: ATM-Dependent and -Independent Dynamics of the Nuclear Phosphoproteome After DNA Damage

A. Bensimon et al.

Quantitative phosphoproteomics suggests kinases other than ATM participate in the initial phase of the cellular response to DNA double-strand breaks.

PROTOCOL: Planar Patch Clamp Approach to Characterize Ionic Currents from Intact Lysosomes

M. Schieder et al.

Glass chips and gentle isolation are the keys to recording channels from intracellular organelles.

SCIENCE CAREERS

www.sciencereers.org/career_magazine

Free Career Resources for Scientists

Serving a Nation's Health

V. Raper

Scientists working in and around the United Kingdom's National Health Service can make a direct impact on public health.

Joining NSF's Culture of Communication

R. Hoover

A rhetorician reports the results of his research on communicating with the National Science Foundation.

SCIENCE TRANSLATIONAL MEDICINE

www.sciencetranslationalmedicine.org

Integrating Medicine and Science

EDITORIAL: Summun Bonum

C. Ash

Scientists and policy-makers must achieve a public consensus about the value of vaccination to individuals and society.

REVIEW: Lost in Translation—Neuropsychiatric Drug Development

R. E. Becker and N. H. Greig

The sluggish pace of new drug development for neuropsychiatric diseases can be partly attributed to faulty clinical trial design.

RESEARCH ARTICLE: Gene from a Psoriasis Susceptibility Locus Primes the Skin for Inflammation

R. Wolf et al.

Psoriasis candidate genes promote susceptibility to skin inflammation and provide new therapeutic targets.

RESEARCH ARTICLE: Maternal Plasma DNA Sequencing Reveals the Genome-Wide Genetic and Mutational Profile of the Fetus

Y. M. D. Lo et al.

Sequencing plasma DNA from a pregnant woman permits prenatal genome-wide scanning for mutations in fetal DNA.

MEETING REPORT: A Crisis of Public Confidence in Vaccines

S. Black and R. Rappuoli

This meeting report summarizes the outcome of a meeting held in July 2010 in Siena, Italy.

SCIENCEPODCAST

www.sciencemag.org/multimedia/podcast

Free Weekly Show

Download the 10 December *Science* Podcast to hear about the cloud-climate feedback, how thinking about food reduces consumption, using DNA to fight fishing fraud, and more.

SCIENCEINSIDER

news.sciencemag.org/scienceinsider

Science Policy News and Analysis

SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals Mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Copyright © 2010 by the American Association for the Advancement of Science. The title SCIENCE is a registered trademark of the AAAS. Domestic individual membership and subscription (51 issues): \$146 (\$74 allocated to subscription). Domestic institutional subscription (51 issues): \$910; Foreign postage extra: Mexico, Caribbean (surface mail) \$55; other countries (air assist delivery) \$85. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request, GST #1254 88122. Publications Mail Agreement Number 1069624. Printed in the U.S.A.

Change of address: Allow 4 weeks, giving old and new addresses and 8-digit account number. **Postmaster:** Send change of address to AAAS, P.O. Box 96178, Washington, DC 20090-6178. **Single-copy sales:** \$10.00 current issue, \$15.00 back issue prepaid includes surface postage; bulk rates on request. **Authorization to photocopy** material for internal or personal use under circumstances not falling within the fair use provisions of the Copyright Act is granted by AAAS to libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that \$20.00 per article is paid directly to CCC, 222 Rosewood Drive, Danvers, MA 01923. The identification code for *Science* is 0036-8075. *Science* is indexed in the *Reader's Guide to Periodical Literature* and in several specialized indexes.



ADVANCING SCIENCE. SERVING SOCIETY



CREDITS (TOP TO BOTTOM): ©VIVEK MENON; IMAGE COURTESY OF THE IMAGE SCIENCE AND ANALYSIS LABORATORY, NASA JOHNSON SPACE CENTER

Assessing Biodiversity Declines

Understanding human impact on biodiversity depends on sound quantitative projection. **Pereira *et al.*** (p. 1496, published online 26 October) review quantitative scenarios that have been developed for four main areas of concern: species extinctions, species abundances and community structure, habitat loss and degradation, and shifts in the distribution of species and biomes. Declines in biodiversity are projected for the whole of the 21st century in all scenarios, but with a wide range of variation. **Hoffmann *et al.*** (p. 1503, published online 26 October) draw on the results of five decades' worth of data collection, managed by the International Union for Conservation of Nature Species Survival Commission. A comprehensive synthesis of the conservation status of the world's vertebrates, based on an analysis of 25,780 species (approximately half of total vertebrate diversity), is presented: Approximately 20% of all vertebrate species are at risk of extinction in the wild, and 11% of threatened birds and 17% of threatened mammals have moved closer to extinction over time. Despite these trends, overall declines would have been significantly worse in the absence of conservation actions.

Making a Point with Metamaterials

A long-predicted electromagnetic excitation, the toroidal moment (or anapole), is associated with toroidal shape and current flow within a structure and has been implicated in nuclear and particle physics. This distinct family of electromagnetic excitations has not been observed directly because they are masked by much stronger electric and magnetic multipoles. **Kaelberer *et al.*** (p. 1510, published online 4 November) have developed a metamaterial structure based on stacked loops of inverted split-ring resonators ("metamolecules") whose response under excitation is consistent with the existence of a toroidal moment. The metamaterial is designed so that both the electric and magnetic dipole moments induced by an incident electromagnetic wave are suppressed, while the toroidal response is isolated and resonantly enhanced to a detectable level.

Fragile Tin Oxide Electrodes

While tin oxide has a high energy density, and would thus make an attractive anode material for a Li-ion battery, it undergoes significant volume changes when Li is intercalated. The large strains cause cracking, pulverization, and a resultant loss of electrical conduction. **Huang *et al.*** (p. 1515; see the Perspective by **Chiang**) used in situ transmission electron microscopy on a single tin oxide nanowire to identify the physical changes that occur during intercalation and observed a moving cloud of dislocations that separated the reacted and unreacted sections. Upon completion of the electrochemical charging, the nanowire showed up to 90% elongation and a 35% increase in diameter.

For the Love of Iron

Iron-loving elements such as Re, Os, Ir, Pt, Rh, Pd, and Au must have been delivered to the upper mantle of Earth, Mars, and the Moon after formation of the planetary cores, because, before that, these elements tended to bond with the core's metallic iron, stripping them from the planetary upper layers. Using Monte Carlo models, **Bottke *et al.*** (p. 1527) show that the relative abundances of iron-loving elements on Earth, Mars, and the Moon can be explained if most of the impacting planetesimals that delivered the elements had sizes extending up to several thousand kilometers. In these circumstances, most of the iron-loving elements would

arrive in a small number of random impacts, the most massive of which hit Earth but not the Moon. Some of these impacts may also have altered Earth's obliquity, produced the Moon's orbital inclination, and delivered water to the Moon's mantle.

Mechanical Transparency

In atomic gases and other solid-state systems with appropriate energy levels, manipulation of the optical properties can be induced with a control pulse, allowing the system to transmit light of specific wavelengths that would otherwise have been absorbed. **Weis *et al.*** (p. 1520, published online 11 November) now report electromagnetically induced transparency in an optomechanical system whereby the coupling of a cavity to a light pulse is used to control the transmission of light through the cavity. This approach may help to allow the engineering of light storage and routing on an optical chip.

Positive Message

Climate warming affects both cloud number and cloud properties, which in turn affect warming itself, creating a cloud-climate feedback that complicates predictions of the amount of warming caused by increasing concentrations of atmospheric carbon dioxide. This feedback has generally been considered to



be positive, but so far we have only a qualitative idea of the effect. **Dessler** (p. 1523; see the news story by **Kerr**) estimated the magnitude of the feedback by analyzing 10 years of satellite data on the flux of radiation through the top of the atmosphere. As expected, the feedback is positive and within the canonical range of estimates of how much warming will occur for a doubling of atmospheric CO₂: 2°C to 4.5°C.

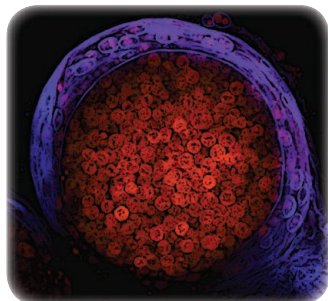
All in the Mind

Pavlov's experiments, in which dogs salivate in anticipation of food, mirror our own imagined experience; that is, thinking about the future consumption of chocolate enhances our desire for it and our motivation to obtain it. After several bites, however, our appetite usually wanes and

Continued on page 1453

Continued from page 1451

the offer of a second bar is less appealing than the first. **Morewedge et al.** (p. 1530) show that the decrease in hedonic response can also be induced by having imagined eating the first bar of chocolate. In comparisons of subjects asked to imagine the repetitive consumption of candy or cheese, they observed a specific drop in the amount consumed when subjects were actually offered the previously imagined foods to eat.



Sex Triangle

The model organism *Dictyostelium discoideum* is a social amoeba that has three sexes, or mating types, that do not resemble those in any other eukaryote studied so far. Any two sexes can form a diploid zygote, which will recruit other haploid cells to form a macrocyst. **Bloomfield et al.** (p. 1533; see the Perspective by **Kessin**) found that sex in this amoeba is determined by several genes at a locus on chromosome 5, with each mating type represented by a different version of the locus. Not all of the genes were directly essential for successful mating, and the

master regulators appeared to be two small soluble intracellular proteins, which control mating types I and III, and a combination of homologs of these two proteins that control mating type II.

Muscle Building

The signaling mechanisms involved in actin filament formation for myofibril formation, which is required for growth factor-induced muscle maturation and hypertrophy, remain unclear. **Takano et al.** (p. 1536; see the Perspective by **Gautel and Ehler**) now show that the mechanism involves the interaction of nebulin and N-WASP. N-WASP is an activator of the Arp2/3 complex, which induces branched actin filaments in nonmuscle cells. The nebulin–N-WASP complex formed in muscle, however, causes nucleation of unbranched actin filaments within myofibrils without the Arp2/3 complex. Nebulin–N-WASP–mediated myofibrillar actin filament formation is required for muscle hypertrophy and might explain a congenital hereditary neuromuscular disorder caused by nebulin gene mutation: nemaline myopathy.

From Blight to Powdery Mildew

Pathogenic effects of microbes on plants have widespread consequences. Witness, for example, the cultural upheavals driven by potato blight in the 1800s. A variety of microbial pathogens continue to afflict crop plants today, driving both loss of yield and incurring the increased costs of control mechanisms. Now, four reports analyze microbial genomes in order to understand better how plant pathogens function (see the Perspective by **Dodds**). **Raffaele et al.** (p. 1540) describe how the genome of the potato blight pathogen accommodates transfer to different hosts. **Spanu et al.** (p. 1543) analyze what it takes to be an obligate biotroph in barley powdery mildew, and **Baxter et al.** (p. 1549) ask a similar question for a natural pathogen of *Arabidopsis*. **Schirawski et al.** (p. 1546) compared genomes of maize pathogens to identify virulence determinants. Better knowledge of what in a genome makes a pathogen efficient and deadly is likely to be useful for improving agricultural crop management and breeding.

Getting HIV Under Control

Approximately 1 in 300 people infected with HIV are HIV “controllers” who are able to maintain long-term control of the virus without medication and who do not progress to AIDS. Uncovering the genetic basis for this ability is of great interest. **Pereyra et al.** (p. 1551, published online 4 November; see the Perspective by **McMichael and Jones**) now present genome-wide association results from patients enrolled in the International HIV Controllers Study. The analysis compared HIV controllers of European, African-American, and Hispanic descent with HIV progressors and found >300 variants that reached genome-wide significance, all of which were in the major histocompatibility class I (HLA) region on chromosome 6. Analysis of the effects of individual amino acids within classical HLA proteins revealed six independently significant residues, five of which lined the peptide-binding groove. Thus, differences in binding to viral peptide antigens by HLA may be the major factors underlying genetic differences between HIV controllers and progressors.



AAAS is here –

increasing diversity in the scientific work force.

AAAS is working to ensure that every student with an aptitude for science, technology, engineering, and mathematics gets an opportunity to pursue a chosen profession, no matter what the challenges. For over 30 years AAAS's ENTRY POINT! program has placed talented, differently abled students in paid internships with leading scientific employers. As a AAAS member your dues support these efforts.

If you're not yet a AAAS member, join us. Together we can make a difference.

To learn more, visit aaas.org/plusyou/entrypoint





Volker ter Meulen is past president of the German Academy of Sciences Leopoldina, Halle (Salle), Germany. E-mail: termeulen@vim.uni-wuerzburg.de.



Günter Stock is president of the Berlin-Brandenburg Academy of Sciences and Humanities, Berlin, Germany. E-mail: stock@bbaw.de.

Cultivating Young Academies

THIS OCTOBER, GERMANY CELEBRATED THE 20TH ANNIVERSARY OF ITS REUNIFICATION. OVER THE past two decades, the country has worked hard to reestablish its leadership in the sciences, investing heavily in R&D. Today, it allocates 2.7% of its gross domestic product toward this end. This commitment has led to the growth of Germany's research institutions, graduate programs, and international collaborations. The government's Exzellenzinitiative, which commits 2.7 billion Euros over 5 years for German universities that are the most active in research, is just one example. Nevertheless, the nation's growing need for well-trained and highly educated people raises the question of whether Germany is doing enough to support its future scientific leaders. The economic downturn threatens to decrease research career opportunities at a time when building scientific capacity to tackle global challenges has become a high priority.

Ten years ago, the German National Academy of Sciences Leopoldina and the Berlin-Brandenburg Academy of Sciences and Humanities recognized that nurturing young scientists would be the key to rebuilding a strong and competitive scientific environment. They created Die Junge Akademie (the Young Academy), an organization intended to harness the resources of both academies in ways that would fertilize research fields with new ideas and bolster career pathways, as well as invigorate older academies by involving the young scientific community in critical policy-related work.

Indeed, for the past decade, the German Young Academy has enabled young scientists to work together across disciplines and geographical boundaries. Many problems that countries share—tackling climate change and environmental degradation, securing new energy and food sources, and promoting health—require answers that will only come from interdisciplinary approaches. The Young Academy has also established itself as an effective voice of the scientific community. Surprisingly, it has sometimes been a more effective advocate for science than the older academies, as reflected by the new Junior Professorships established in almost all German universities. The Young Academy has also become a principal point of contact for other organizations involved in higher education and research. Young Academy members are involved in public events that promote dialogue between science and society, inspiring scientists to engage more energetically with the public and media. And the members are increasingly active internationally, facilitating an exchange of ideas between researchers, business, and policy-makers, and stimulating the formation of similar young academies in other nations.

In the past few years, there has been a substantial increase in successful applications by young German scientists for support from the European Research Council. This positive trend can probably be attributed to multiple efforts that support young scientists in Germany. But the success of the Young Academy should be judged much more broadly. The foundation of the Young Academy was designed “to help young scientists develop a fuller view of the scientific universe; to give this view human meaning by associating it with faces and friends; to remind them that science is an endeavour of freely organized minds; and to urge them to take this spirit to the scientific community at large.”*

In practice, the Young Academy has more than fulfilled this vision. It has given young academics an effective voice in the political, scientific, and public dialogue about their future career pathways. It has stimulated an awareness of and commitment to interdisciplinary working relationships, which are indispensable for tackling future challenges and needs. And it has generated a new culture of cooperation between scientists. The idea of a Young Academy has been spreading around the globe, because every nation must support and develop its younger scientists, promoting their national and international mobility, competitiveness, and leadership potential.**

— Volker ter Meulen and Günter Stock

10.1126/science.1200095





ECOLOGY

Narrow Niches and Nowhere to Go

Studies of the changing distributions of plant species under contemporary climate change tend to not take into account the often quite specialized requirements of plants for soil composition, pollinators, and regeneration conditions. A good example is plant species found on serpentine soils, which are generally restricted geographically. Damschen *et al.* revisited the rich but narrowly restricted floras on serpentine in the Siskiyou Mountains in the northwest United States, first studied 60 years ago by the ecologist Robert Whittaker. They found striking changes in the species composition of herbaceous communities, with the strongest declines in the more-specialist serpentine species, which have no suitable neighboring habitat to migrate to. The plant communities are becoming more characteristic of drier, warmer habitats, and with fewer, more widely tolerant species. Besides showing how climate change may pose special risks to the floras of hot spots of endemic species, this work illustrates the increasing power of long-term ecological records in illuminating the effects of climate change on biota. — AMS

Ecology **91**, 3609 (2010).

MOLECULAR BIOLOGY

Less Expression from X

Sex is genetically determined in many organisms and has often involved the evolution of specialized sex chromosomes—the X and Y chromosomes in mammals, for example. The sex chromosomes originally evolved from a pair of normal chromosomes, or autosomes. To balance gene dosage between mammalian female (XX) cells and male (XY) cells, one of the pair of female XX chromosomes is inactivated, a process known as X inactivation. Because dosage compensation can involve inactivating or reducing gene expression on the X chromosome, it has been hypothesized by Susumu Ohno that, during evolution, gene expression on an individual X must have been boosted chromosome-wide by a factor of 2 to balance expression with the (paired) autosomes, giving an X:AA expression ratio of 1.

Xiong *et al.* have used publicly available RNA sequencing data, which they demonstrate is more sensitive than equivalent microarray-based analyses, to measure the X:AA expression ratio across

several mouse and human tissues. They find that the ratio is consistently lower than 1, and the average falls close to 0.5, which is inconsistent with Ohno's hypothesis. Proteomic data also support an expression ratio of ~0.5 in mice and worms. The apparent absence of expression equalization between the sex chromosomes and autosomes suggests that new models are needed to explain dosage compensation. — GR

Nat. Genet. **42**, 1043 (2010).

MATERIALS SCIENCE

A Stressful Situation

We typically think of fluids as materials that flow when stressed. Water and honey, for instance, can both be poured, even though the flows occur on different time scales because of differences in viscosity. However, some fluids, such as mayonnaise or tomato paste, show yield properties that are typically associated with a solid. When undisturbed, they withstand the force of gravity and do not flow out of a container, and when gently pushed and released they elastically return

to their original shape. Reliable measurements of the yield stress are difficult to obtain and can vary considerably from one technique to the next. Fall *et al.* measured the rheological behavior of emulsions of surfactant-stabilized oil droplets in water. For this simple fluid, the yield stresses measured through increasing or decreasing shear stress sweeps, i.e., in going from/to a solid or static state to/from a liquid or dynamic one, were the same. However, most real yield-stress fluids are thixotropic: Induced flow temporarily changes the internal structure of the fluid, so that the viscosity also depends on the shear history. The authors achieved this property by adding clay particles to their emulsions, which linked together the oil droplets. In this composition, the transition is complicated by the constant rejuvenation and breakdown of the aggregates under changing flow conditions. However, the authors found that by measuring the lowest stress under which steady-state flow occurs, they could define a dynamic yield stress, which also provides valid measurements for simple fluids. — MSL

Phys. Rev. Lett. **105**, 225502 (2010).

CREDITS: ELLEN DAMSCHEN

APPLIED PHYSICS

Hitting the Spot in 3D

High-density optical storage media, high-resolution spectroscopy, and surface imaging rely on the ability to focus a beam of light to as small a spot as possible. There are limitations, though, to how small a spot can be made. Certain tricks can be used, such as manipulating the phase of the incident light, so that interference effects can bring the size down close to and even beyond the diffraction limit. Because the light is incident from just one side, such tricks tend to concentrate on focusing the spot only in the two-dimensional (2D) plane and ignore the out-of-focus features along the optic axis. To provide focusing in a small volume, Mudry *et al.* address the asymmetry of light incidence by placing a mirror behind the focal plane. Their trick is to apply a time-reversal process, which just means that the illumination wavefront is shaped with a spatial light modulator so that the incident and reflected beams converge at the same point. Such a technique of focusing light to a small volume should be useful in 3D imaging applications. — ISO
Phys. Rev. Lett. **105**, 203903 (2010).

ASTRONOMY

X-ray Vision

In 2008, the Fermi Large Area Telescope detected a pulsar based solely on its gamma-ray pulsations. Now, using the XMM-Newton space-based telescope, Lin *et al.* and Caraveo *et al.* have revealed that this source, PSR J0007+7303, also pulsates in x-rays. Pulsars are magnetized, rapidly rotating neutron stars; like lighthouses, they appear to flash periodically as their beams of electromagnetic radiation cross our line of sight. For many decades, the vast majority of pulsars astronomers knew about were detected through their radio emission. PSR J0007+7303 was only the second radio-quiet pulsar to be detected in gamma rays; now it is also the second radio-quiet gamma-ray pulsar to show x-ray pulsations.

These observations help build a more complete picture of this pulsar. The x-rays are believed to stem from both the heat of the neutron star's surface and from relativistic particles in the star's magnetosphere, which are also the origin of the gamma rays. In the case of PSR J0007+7303, the heat component appears to dominate; the



x-ray pulsations possibly originate from a hot spot on the neutron star's surface, which is heated by return currents from the magnetosphere. — MJC

Astrophys. J. **725**, L1; L6 (2010).

IMMUNOLOGY

You Are What You Eat

Oligosaccharides modified by the addition of a sialic acid to lactose are a major component of milk. These sialylated sugars may act to shape the composition of the gut microflora in neonatal mammals, because they can act as a food source for colonizing bacteria. By comparing mice nursed by wild-type mothers to mice nursed by mothers deficient in sialyl(α 2,3)lactose, Fuhrer *et al.* found that the absence of just this one milk sugar reduced the sensitivity of the mice to acute colitis induced several weeks after weaning. Mice fed sialyl(α 2,3)lactose-deficient milk had an altered composition of their intestinal microflora as compared to mice fed normal milk, suggesting that differences in bacterial colonization may have shaped the later susceptibility to gut injury. These results suggest that besides providing passive immunity to the newborn, milk components may act to shape gut colonization by commensal microflora and thereby affect subsequent immune responses, for better or for worse. — KLM

J. Exp. Med. **10.1084/jem.20101098** (2010).

MOLECULAR BIOLOGY

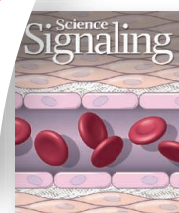
RNAs Amplify

Eukaryotic unicellular ciliates contain two nuclei, a micronucleus and a macronucleus. After sexual reproduction, the macronucleus is derived from the micronucleus through DNA rearrangements, which include gene processing, the removal of all noncoding material, and the amplification of the exons of coding genes. Heyse *et al.* and Nowacki *et al.* now investigate the influence of RNAs on gene amplification within the macronucleus of the ciliates *Stylonychia* and *Oxytricha*, respectively. Both groups found that DNA copy numbers decreased when additional complementary RNAs were added and increased upon the addition of anti-complementary RNAs. Future

studies should describe the underlying mechanism behind these results and determine whether RNA-mediated DNA amplifications and reductions are limited to the ciliates or also occur in other organisms. — LMZ

Proc. Natl. Acad. Sci. U.S.A. **10.1073/pnas.1009284107**; **10.1073/pnas.1012236107** (2010).

Call for Papers



Science Signaling

Science Signaling, from the publisher of **Science**, AAAS, features top-notch, peer-reviewed, original research weekly. Submit your manuscripts in the following areas of cellular regulation:

- Biochemistry
- Bioinformatics
- Cell Biology
- Development
- Immunology
- Microbiology
- Molecular Biology
- Neuroscience
- Pharmacology
- Physiology and Medicine
- Systems Biology

Subscribing to **Science Signaling** ensures that you and your lab have the latest cell signaling resources. For more information visit **www.ScienceSignaling.org**

Chief Scientific Editor

Michael B. Yaffe, M.D., Ph.D.
Associate Professor, Department of Biology
Massachusetts Institute of Technology

Editor

Nancy R. Gough, Ph.D.
AAAS

Submit your research at:
**www.sciencesignaling.org/
about/help/research.dtl**

Science Signaling



**1200 New York Avenue, NW
Washington, DC 20005**
Editorial: 202-326-6550, FAX 202-289-7562
News: 202-326-6581, FAX 202-371-9227
**Bateman House, 82-88 Hills Road
Cambridge, UK CB2 1LQ**
+44 (0) 1223 326500, FAX +44 (0) 1223 326501

SUBSCRIPTION SERVICES For change of address, missing issues, new orders and renewals, and payment questions: 866-434-AAAS (2227) or 202-326-6417, FAX 202-842-1065. Mailing addresses: AAAS, P.O. Box 96178, Washington, DC 20090-6178 or AAAS Member Services, 1200 New York Avenue, NW, Washington, DC 20005

INSTITUTIONAL SITE LICENSES please call 202-326-6755 for any questions or information

REPRINTS: Author Inquiries 800-635-7181

Commercial Inquiries 803-359-4578

PERMISSIONS 202-326-7074, FAX 202-682-0816

MEMBER BENEFITS AAAS/Barnes&Noble.com bookstore www.aaas.org/bn; AAAS Online Store www.apisource.com/aaas/ code MKB6; AAAS Travels: Betchart Expeditions 800-252-4910; Apple Store www.apple.com/epppstore/aaas; Bank of America MasterCard 1-800-833-6262 priority code FAA3YU; Cold Spring Harbor Laboratory Press Publications www.cshlpress.com/affiliates/aaas.htm; GEICO Auto Insurance www.geico.com/landingpage/go51.htm?logo=17624; Hertz 800-654-2200 CDP#343457; Office Depot https://bsd.officedepot.com/portallogin.do; Seabury & Smith Life Insurance 800-424-9883; Subaru VIP Program 202-326-6417; VIP Moving Services www.vipmayflower.com/domestic/index.html; Other Benefits: AAAS Member Services 202-326-6417 or www.aaasmember.org.

science_editors@aaas.org (for general editorial queries)
science_letters@aaas.org (for queries about letters)
science_reviews@aaas.org (for returning manuscript reviews)
science_bookrevs@aaas.org (for book review queries)

Published by the American Association for the Advancement of Science (AAAS), *Science* serves its readers as a forum for the presentation and discussion of important issues related to the advancement of science, including the presentation of minority or conflicting points of view, rather than by publishing only material on which a consensus has been reached. Accordingly, all articles published in *Science*—including editorials, news and comment, and book reviews—are signed and reflect the individual views of the authors and not official positions of view adopted by AAAS or the institutions with which the authors are affiliated.

AAAS was founded in 1848 and incorporated in 1874. Its mission is to advance science, engineering, and innovation throughout the world for the benefit of all people. The goals of the association are to: enhance communication among scientists, engineers, and the public; promote and defend the integrity of science and its use; strengthen support for the science and technology enterprise; provide a voice for science on societal issues; promote the responsible use of science in public policy; strengthen and diversify the science and technology workforce; foster education in science and technology for everyone; increase public engagement with science and technology; and advance international cooperation in science.

INFORMATION FOR AUTHORS

See pages 352 and 353 of the 15 January 2010 issue or access www.sciencemag.org/about/authors

EDITOR-IN-CHIEF **Bruce Alberts**

EXECUTIVE EDITOR

Monica M. Bradford

NEWS EDITOR

Colin Morman

MANAGING EDITOR, RESEARCH JOURNALS **Katrina L. Kelner**
DEPUTY EDITORS **R. Brooks Hanson, Barbara R. Jasny, Andrew M. Sugden**

EDITORIAL SENIOR EDITORS/COMMENTARY Lisa D. Chong, Brad Wible; **SENIOR EDITORS** Gilbert J. Chin, Pamela J. Hines, Paula A. Kiberstis (Boston), Marc S. Lavine (Toronto), Beverly A. Purnell, L. Bryan Ray, Guy Riddiough, H. Jesse Smith, Phillip D. Szurimi (Tennessee), Valda Vinson, Jake S. Yeston; **ASSOCIATE EDITORS** Kristen L. Mueller, Jelena Stajic, Sacha Vignieri, Nicholas S. Wigginton, Laura M. Zahn (San Diego); **BOOK REVIEW EDITOR** Sherman J. Suter; **ASSOCIATE LETTERS EDITOR** Jennifer Sills; **EDITORIAL MANAGER** Cara Tate; **SENIOR COPY EDITORS** Jeffrey E. Cook, Cynthia Howe, Harry Jach, Lauren Kmeic, Barbara P. Ordway, Trista Wagoner; **COPY EDITOR** Chris Filiatreau; **EDITORIAL COORDINATORS** Carolyn Kyle, Beverly Shields; **PUBLICATIONS ASSISTANTS** Ramatoulaye Diop, Joi S. Granger, Emily Guise, Jeffrey Hearn, Michael Hicks, Lisa Johnson, Scott Miller, Jerry Richardson, Jennifer A. Seibert, Brian White, Anita Wynn; **EDITORIAL ASSISTANTS** Emily C. Horton, Patricia M. Moore, Marlene Weinberg; **EXECUTIVE ASSISTANT** Alison Crawford; **ADMINISTRATIVE SUPPORT** Maryrose Madrid; **EDITORIAL FELLOW** Melissa R. McCartney

EDITORIAL DIRECTOR, WEB AND NEW MEDIA Stewart Wills; **SENIOR WEB EDITOR** Tara S. Marathe; **WEB EDITOR** Robert Frederick; **RESEARCH ASSOCIATE** Corinna Chong; **WEB DEVELOPMENT MANAGER** Martyn Green; **WEB DEVELOPER** Andrew Whitesell; **INTERN** Sophia Cai

NEWS DEPUTY NEWS EDITORS Robert Coontz, David Grimm (Online), Eliot Marshall, Jeffrey Mervis, Leslie Roberts; **CONTRIBUTING EDITORS** Elizabeth Colotta, Polly Shulman; **NEWS WRITERS** Yudhijit Bhattacharjee, Adrian Cho, Jennifer Czernin, Jocelyn Kaiser, Richard A. Kerr, Eli Kintisch, Greg Miller, Elizabeth Pennisi, Lauren Schenckman, Robert F. Service (Pacific NW), Erik Stokstad; **WEB DEVELOPER** Daniel Berger; **INTERN** Kristen Minogue; **CONTRIBUTING CORRESPONDENTS** Jon Cohen (San Diego, CA), Daniel Ferber, Ann Gibbons, Sam Kean, Andrew Lawler, Mitch Leslie, Charles C. Mann, Virginia Morell, Gary Taubes; **COPY EDITORS** Linda B. Felaco, Melvin Gatling, Melissa Raimondi; **ADMINISTRATIVE SUPPORT** Scherraine Mack; **BUREAUS** San Diego, CA: 760-942-3252, FAX 760-942-4979; Pacific Northwest: 503-963-1940

PRODUCTION DIRECTOR Wendy K. Shank; **ASSISTANT MANAGER** Rebecca Doshi; **SENIOR SPECIALISTS** Steve Forrester, Chris Redwood, Anthony Rosen; **PRELIGHT DIRECTOR** David M. Tompkins; **MANAGER** Marcus Spiegler; **SPECIALIST** Jason Hillman

ART DIRECTOR Yael Fitzpatrick; **ASSOCIATE ART DIRECTOR** Laura Creveling; **SENIOR ILLUSTRATORS** Chris Bickel, Katharine Suttif; **ILLUSTRATOR** Yana Hammond; **SENIOR ART ASSOCIATES** Holly Bishop, Preston Huey, Nayomi Kevitiyagala; **ART ASSOCIATES** Kay Engman, Matthew Twombly; **PHOTO EDITOR** Leslie Blizard

SCIENCE INTERNATIONAL

EUROPE (science@science-int.co.uk) **EDITORIAL: INTERNATIONAL MANAGING EDITOR** Andrew M. Sugden; **SENIOR EDITOR/COMMENTARY** Julia Fahrenkamp-Uppenbrink; **SENIOR EDITORS** Caroline Ash, Stella M. Hurtle, Ian S. Osborne, Peter Stern; **ASSOCIATE EDITOR** Maria Cruz; **LOCUM EDITOR** Helen Pickersgill; **EDITORIAL SUPPORT** Samantha Hogg, Alice Whaley; **ADMINISTRATIVE SUPPORT** John Cannell, Janet Clements, Louise Hartwell; **NEWS: EUROPE NEWS EDITOR** John Travis; **DEPUTY NEWS EDITOR** Daniel Clerly; **CONTRIBUTING CORRESPONDENTS** Michael Balter (Paris), John Bohnannon (Vienna), Martin Enserink (Amsterdam and Paris), Gretchen Vogel (Berlin); **INTERN** Jennifer Carpenter

LATIN AMERICA CONTRIBUTING CORRESPONDENT Antonio Regalado

ASIA Japan office: Asca Corporation, Tomoko Furusawa, Rustic Bldg. 7F, 77 Tenjin-cho, Shinjuku-ku, Tokyo 162-0808, Japan; +81 3 6802 4616, FAX +81 3 6802 4615, inquiry@sciencemag.jp; **ASIA NEWS EDITOR** Richard Stone (Beijing: rstone@aaas.org); **CONTRIBUTING CORRESPONDENTS** Dennis Normile [Japan: +81 (0) 3 3391 0630, FAX +81 (0) 3 5936 3531; dnornile@gol.com]; Hao Xin [China: cindyhao@gmail.com]; Pallava Bagla [South Asia: +91 (0) 11 2271 2896; pbagla@vsnl.com]

EXECUTIVE PUBLISHER **Alan I. Leshner**

PUBLISHER **Beth Rosner**

FULFILLMENT SYSTEMS AND OPERATIONS (membership@aaas.org); **DIRECTOR** Waylon Butler; **CUSTOMER SERVICE SUPERVISOR** Pat Butler; **SPECIALISTS** Latoya Casteel, LaVonda Crawford, Vicki Linton, April Marshall; **DATA ENTRY SUPERVISOR** Cynthia Johnson; **SPECIALISTS** Shirlene Hall, Tarrika Hill, William Jones

BUSINESS OPERATIONS AND ADMINISTRATION DIRECTOR Deborah Rivera-Wienhold; **BUSINESS SYSTEMS AND FINANCIAL ANALYSIS DIRECTOR** Randy Yi; **MANAGER, BUSINESS ANALYSIS** Eric Knott; **MANAGER, BUSINESS OPERATIONS** Jessica Tierney; **FINANCIAL ANALYSTS** Priti Pamnani, Celeste Troxler; **RIGHTS AND PERMISSIONS: ADMINISTRATOR** Emilie David; **ASSOCIATE** Elizabeth Bandler; **MARKETING DIRECTOR** Ian King; **MARKETING MANAGERS** Allison Pritchard, Alison Chandler, Julianne Wielga; **MARKETING ASSOCIATES** Aimee Aponte, Mary Ellen Crowley, Wendy Wise; **SENIOR MARKETING EXECUTIVE** Jennifer Reeves; **DIRECTOR, SITE LICENSING** Tom Ryan; **DIRECTOR, CORPORATE RELATIONS** Eileen Bernadette Moran; **PUBLISHER RELATIONS, eRESOURCES SPECIALIST** Kiki Forsythe; **SENIOR PUBLISHER RELATIONS SPECIALIST** Catherine Holland; **PUBLISHER RELATIONS, EAST COAST** Phillip Smith; **PUBLISHER RELATIONS, WEST COAST** Philip Tsolakis; **FULFILLMENT SUPERVISOR** Iquo Edim; **FULFILLMENT COORDINATOR** Carrie MacDonald, Destiny Pinson; **MARKETING MANAGER** Christina Schlecht; **MARKETING ASSOCIATE** Laura Tutino; **ELECTRONIC MEDIA: MANAGER** Elizabeth Harman; **PROJECT MANAGER** Trista Snyder; **ASSISTANT MANAGER** Lisa Stanford; **SENIOR PRODUCTION SPECIALISTS** Ryan Atkins, Christopher Coleman, **COMPUTER SPECIALIST** Walter Jones, Kai Zhang; **PRODUCTION SPECIALISTS** Antoinette Hodal, Michele Johnston, Kimberly Oster; **DIRECTOR, WEB AND NEW MEDIA** Will Collins

ADVERTISING DIRECTOR, WORLDWIDE AD SALES Bill Moran

COMMERCIAL EDITOR Sean Sanders: 202-326-6430

ASSISTANT COMMERCIAL EDITOR Tianna Hicklin 202-326-6463

PROJECT DIRECTOR, OUTREACH Brianna Blaser

PRODUCT (science_advertising@aaas.org); **MIDWEST** Rick Bongiovanni: 330-405-7080, FAX 330-405-7081; **EAST COAST/E. CANADA** Laura Faraday: 508-747-9395, FAX 617-507-8189; **WEST COAST/W. CANADA** Lynne Stickrod: 415-931-9782, FAX 415-520-6940; **UK/EUROPE/ASIA** Roger Goncalves: TEL/FAX +41 43 243 1358; **JAPAN** ASCA Corporation, Nanako Ide +81 (0) 3 6802 4616, FAX +81 (0) 3 6802 4615; ads@sciencemag.jp; **SENIOR TRAFFIC ASSOCIATE** Deandra Simms

WORLDWIDE ASSOCIATE DIRECTOR OF SCIENCE CAREERS Tracy Holmes: +44 (0) 1223 326525, FAX +44 (0) 1223 326532

CLASSIFIED (advertise@sciencecareers.org); **U.S.: MIDWEST/WEST COAST/SOUTH CENTRAL/CANADA** Tina Burks: 202-326-6577; **EAST COAST/INDUSTRY** Elizabeth Early: 202-326-6578; **SALES ADMINISTRATOR:** Marci Gallun; **SALES COORDINATORS** Rohan Edmonson, Shirley Young; **EUROPE/ROW SALES:** Susanne Kharraz, Dan Pennington, Alex Palmer; **SALES ASSISTANT** Lisa Patterson; **JAPAN** ASCA Corporation, Jie Chin +81 (0) 3 6802 4616, FAX +81 (0) 3 6802 4615; careerads@sciencemag.jp; **ADVERTISING SUPPORT MANAGER** Karen Foote: 202-326-6740; **ADVERTISING PRODUCTION OPERATIONS MANAGER** Deborah Tompkins; **SENIOR PRODUCTION SPECIALIST/GRAPHIC DESIGNER** Amy Hardcastle; **PRODUCTION SPECIALIST** Yuse Lajiminmuh; **SENIOR TRAFFIC ASSOCIATE** Christine Hall

AAAS BOARD OF DIRECTORS RETIRING PRESIDENT, CHAIR Peter C. Agre; **PRESIDENT** Alice Huang; **PRESIDENT-ELECT** Nina Fedoroff; **TREASURER** David E. Shaw; **CHIEF EXECUTIVE OFFICER** Alan I. Leshner; **BOARD** Linda P. B. Katehi, Nancy Knowlton, Stephen Mayo, Cherry A. Murray, Julia M. Phillips, Sue V. Rosser, David D. Sabatini, Thomas A. Woolsey



ADVANCING SCIENCE. SERVING SOCIETY

SENIOR EDITORIAL BOARD

John I. Brauman, *Chair, Stanford Univ.*
Richard Losick, *Harvard Univ.*
Linda Partridge, *Univ. College London*
Michael S. Turner, *University of Chicago*

BOARD OF REVIEWING EDITORS

Adriano Aguzzi, *Univ. Hospital Zürich*
Takuzo Aida, *Univ. of Tokyo*
Sonia Altizer, *Univ. of Georgia*
David Altschuler, *Broad Institute*
Arturo Alvarez-Buylla, *Univ. of California, San Francisco*
Richard Amasino, *Univ. of Wisconsin, Madison*
Angelika Amon, *MIT*
Kathryn Anderson, *Memorial Sloan-Kettering Cancer Center*
Siv G. E. Andersson, *Uppsala Univ.*
Peter Andolfatto, *Princeton Univ.*
Meinrat O. Andreae, *Max Planck Inst., Mainz*
John A. Bargh, *Yale Univ.*
Ben Barres, *Stanford Medical School*
Marisa Bartolomeo, *Univ. of Penn. School of Med.*
Jordi Bascompte, *Estación Biológica de Doñana, CSIC*
Facundo Batista, *London Research Inst.*
Ray H. Baughman, *Univ. of Texas, Dallas*
David Baum, *Univ. of Wisconsin*
Yasmine Belkaid, *NIAID, NIH*
Stephen J. Benkovic, *Penn. State Univ.*
Gregory C. Berzox, *Stanford Univ.*
Tom Bisseling, *Wageningen Univ.*
Mina Bissell, *Lawrence Berkeley National Lab*
Peer Bork, *EMBL*
Robert W. Boyd, *Univ. of Rochester*
Paul M. Brakefield, *Leiden Univ.*
Christian Büchel, *Universitätsklinikum Hamburg-Eppendorf*
Joseph A. Burns, *Cornell Univ.*
William P. Buttz, *Population Reference Bureau*
Mats Carlsson, *Univ. of Oslo*
Mildred Cho, *Stanford Univ.*
David Clapham, *Children's Hospital, Boston*
David Clary, *Oxford University*
J. M. Claverie, *CHRS, Marseille*
Jonathan D. Cohen, *Princeton Univ.*
Andrew Cossins, *Univ. of Liverpool*
Alan Cowman, *Walter & Eliza Hall Inst.*
Robert H. Crabtree, *Yale Univ.*
Wolfgang Cramer, *Potsdam Inst. for Climate Impact Research*

F. Fleming Crim, *Univ. of Wisconsin*
Jeff L. Dangl, *Univ. of North Carolina*
Tom Daniel, *Univ. of Washington*
Stamatis Dehaene, *Collège de France*
Emmanouil T. Dermitzakis, *Univ. of Geneva Medical School*
Robert Desimone, *MIT*
Claude Desplan, *New York Univ.*
Ap Dijksterhuis, *Radboud Univ. of Nijmegen*
Dennis Discher, *Univ. of Pennsylvania*
Scott C. Doney, *Woods Hole Oceanographic Inst.*
Jennifer A. Doudna, *Univ. of California, Berkeley*
Julian Downward, *Cancer Research UK*
Bruce Dunn, *Univ. of California, Los Angeles*
Christopher Dye, *WHO*
Michael B. Elowitz, *Calif. Inst. of Technology*
Gerhard Ertl, *Fritz-Haber-Institut, Berlin*
Barry Everitt, *Univ. of Cambridge*
Paul G. Falkowski, *Rutgers Univ.*
Ernst Fehr, *Univ. of Zurich*
Tom Feuchel, *Univ. of Copenhagen*
Alain Fischer, *INSERM*
Wulfam Gerstner, *EPFL Lausanne*
Karl-Heinz Glassmeier, *Inst. for Geophysics & Extraterrestrial Physics*
Charles Godfrey, *Univ. of Oxford*
Diane Griffin, *Johns Hopkins Bloomberg School of Public Health*
Christian Haas, *Ludwig Maximilians Univ.*
Steven Hahn, *Fritz-Hutchinson Cancer Research Center*
Gregory J. Hanson, *Cold Spring Harbor Lab.*
Niels Hansen, *Technical Univ. of Denmark*
Dennis L. Hartmann, *Univ. of Washington*
Chris Hawkesworth, *Univ. of St Andrews*
Martin Heimann, *Max Planck Inst., Jena*
James A. Hendler, *Rensselaer Polytechnic Inst.*
Janet G. Hering, *Swiss Fed. Inst. of Aquatic Science & Technology*
Ray Hilborn, *Univ. of Washington*
Michael E. Himmel, *National Renewable Energy Lab.*
Kei Hirose, *Tokyo Inst. of Technology*
Ove Hoegh-Guldberg, *Univ. of Queensland*
David Holden, *Imperial College*
Laura Hooper, *UT Southwestern Medical Ctr at Dallas*
Ronald R. Hoy, *Cornell Univ.*
Jeffrey A. Hubbell, *EPFL Lausanne*
Steven Jacobsen, *Univ. of California, Los Angeles*
Peter Jonas, *Universität Freiburg*
Barbara B. Kahn, *Harvard Medical School*

Daniel Kahne, *Harvard Univ.*
Bernhard Keimer, *Max Planck Inst., Stuttgart*
Robert Kingston, *Harvard Medical School*
Hanna Kokko, *Univ. of Helsinki*
Alberto R. Kornblith, *Univ. of Buenos Aires*
Leonid Kruglyak, *Princeton Univ.*
Lee Kump, *Penn State Univ.*
Mitchell A. Lazar, *Univ. of Pennsylvania*
David Lazer, *Harvard Univ.*
Virginia Lee, *Univ. of Pennsylvania*
Ottoline Leyser, *Univ. of New York*
Olle Lindvall, *Univ. Hospital, Lund*
Marcia C. Linn, *Univ. of California, Berkeley*
John Lis, *Cornell Univ.*
Richard Losick, *Harvard Univ.*
Jonathan Losos, *Harvard Univ.*
Ke Lu, *Chinese Acad. of Sciences*
Laura Machesky, *CRUK Beatson Inst. for Cancer Research*
Andrew P. Mackenzie, *Univ. of St Andrews*
Anne Margun, *Univ. of St Andrews*
Oscar Marin, *CSIC & Univ. Miguel Hernández*
Charles Marshall, *Univ. of California, Berkeley*
Martin M. Matzuk, *Baylor College of Medicine*
Graham Medley, *Univ. of Warwick*
Virginia Miller, *UNC, Chapel Hill*
Yasushi Miyashita, *Univ. of Tokyo*
Richard Morris, *Univ. of Edinburgh*
Edward Moser, *Norwegian Univ. of Science and Technology*
Jonas Murraro, *MRC Lab. of Molecular Biology*
Naoto Nagao, *Univ. of Tokyo*
James Nelson, *Stanford Univ. School of Med.*
Timothy W. Nilsen, *Case Western Reserve Univ.*
Pär Nordlund, *Karolinska Inst.*
Helga Nowotny, *European Research Advisory Board*
Stuart H. Orkin, *Dana-Farber Cancer Inst.*
Christine Ortiz, *MIT*
Elinor Ostrom, *Indiana Univ.*
Andrew Oswald, *Univ. of Warwick*
Jonathan T. Overbeck, *Univ. of Arizona*
P. David Pearson, *Univ. of California, Berkeley*
John Pendry, *Imperial College*
Reginald M. Penner, *Univ. of California, Irvine*
John H. J. Petrij, *Memorial Sloan-Kettering Cancer Center*
Simon Philpott, *Univ. of Florida*
Philippe Poulin, *CNRS*
Colin Renfrew, *Univ. of Cambridge*
Trevor Robbins, *Univ. of Cambridge*
Barbara A. Romanowicz, *Univ. of California, Berkeley*

Jens Rostrup-Nielsen, *Haldor Topsøe*
Edward M. Rubin, *Lawrence Berkeley National Lab*
Shimon Sakaguchi, *Kyoto Univ.*
Michael I. Sanderson, *Univ. of Arizona*
Jürgen Sandkühner, *Medical Univ. of Vienna*
Randy Seeley, *Univ. of Cincinnati*
Christine Seidman, *Harvard Medical School*
Vladimir Shalae, *Purdue Univ.*
Joseph Silk, *Univ. of Oxford*
Montgomery Slatkin, *Univ. of California, Berkeley*
Davor Solter, *Inst. of Medical Biology, Singapore*
Allan C. Spradling, *Carnegie Institution of Washington*
Jonathan Sprent, *Garvan Inst. of Medical Research*
Elsbeth Stern, *ETH Zürich*
Yoshiko Takahashi, *Mara Inst. of Science and Technology*
Jürg Tschopp, *Univ. of Lausanne*
Herbert Virgin, *Washington Univ.*
Bert Vogelstein, *Johns Hopkins Univ.*
Cynthia Volkert, *Univ. of Göttingen*
Bruce D. Walker, *Harvard Medical School*
Ian Walmsey, *Univ. of Oxford*
Christopher A. Walsh, *Harvard Medical School*
David A. Wardle, *Swedish Univ. of Agric Sciences*
Colin Watts, *Univ. of Dundee*
Detlef Weigel, *Max Planck Inst., Tübingen*
Jonathan Weissman, *Univ. of California, San Francisco*
Sue Wessler, *Univ. of Georgia*
Ian A. Wilson, *The Scripps Res. Inst.*
Timothy D. Wilson, *Univ. of Virginia*
Xiaoliang Sunney Xie, *Harvard Univ.*
John R. Yates III, *The Scripps Res. Inst.*
Jan Zaanen, *Leiden Univ.*
Mayana Zatz, *University of Sao Paulo*
Jonathan Zehr, *Ocean Sciences*
Huda Zoghbi, *Baylor College of Medicine*
Maria Zuber, *MIT*

BOOK REVIEW BOARD

John Aldrich, *Duke Univ.*
David Bloom, *Harvard Univ.*
Angela Croom, *Princeton Univ.*
Richard Swedner, *Univ. of Chicago*
Ed Wasserman, *DuPont*
Lewis Wolpert, *Univ. College London*



Superhot, Live, and Just a Click Away

Fancy yourself an armchair fusion scientist? Forget the half-built tokamak in your garage; now you can experiment with a real fusion reactor called GOLEM and watch the results live via the Web.

Billy Huang, a doctoral student at the Culham Centre for Fusion Energy near Oxford, U.K., and other students at a summer school at Prague's Institute of Plasma Physics (IPP) wanted to make fusion science more widely accessible. They teamed up with Vojtěch Svoboda, an engineer at the Czech Technical University in Prague, who was rigging up remote controls for GOLEM, a small tokamak there. On 1 December, they opened the tokamak for business (<http://tokamakglobal.com>).

GOLEM, built in the early 1960s at Moscow's Kurchatov Institute, was one of the world's first working tokamaks. Although tiny compared with today's reactors, its hydrogen plasmas reach approximately 1,000,000°C and can produce a small amount of energy by fusing nuclei.

Anyone with a physics background, the project organizers say, can register and request an experiment on GOLEM. On its first day, 38 enthusiasts from 10 countries requested a total of 81 fusion shots. Who knows, Huang says, "We could get some interesting results."

'Nature and Nurture' Researchers Win Prize

Husband-and-wife team Avshalom Caspi and Terrie Moffitt's research on how biology influences behavior has often raised controversy (*Science*, 26 June 2009, p. 1628). Now it has earned them 1 million Swiss francs: At a ceremony in Zurich last Friday, the pair took home the Klaus J. Jacobs Research Prize.

Caspi and Moffitt, who both have joint appointments at Duke University in Durham, North Carolina, and King's College London, have used a longitudinal study of New Zealanders to link genetic mutations with violence and depression while showing that environmental factors, such as childhood abuse or stress, come crucially into play (*Science*, 2 August 2002,

p. 851). That research opened up "whole new lines of work" in how genetics and environment interact, says Anne Petersen, a biobehavioral researcher at the University of Michigan, Ann Arbor, who headed the jury for the prize. "Too often people say we can't really show [that relationship]," she says. Caspi and Moffitt "keep demonstrating that you can."

Survivor: Grant Money

Millions of people across the Arab world tuned in 28 November for the final episode of a reality television show. But the contestants on Qatar's *Stars of Science* weren't vying for pop stardom or trying to steal one another's spouses on a tropical island. Instead, they were young Arab scientists competing for grant money.

Sixteen researchers out of 7000 applicants were relocated to custom laboratories in Doha, where they worked on their projects for months as the cameras rolled. The top prize, a \$300,000 grant, went to Sadek

Qassim, a 26-year-old chemical engineer from Kuwait, for his "Alchemist," an automated system for testing petroleum samples. Three runners-up—swimming goggles that record real-time physiological data, a motorized walker for the elderly, and a versatile robotic joint—received smaller grants.

The show "has been a big hit throughout the Arab region," says Farouk El-Baz, an Egyptian geophysicist at Boston University and one of the judges. Seeing scientific role models on Arab television, he says, "gives a boost to the self-



Sadek Qassim and the "Alchemist" (inset).

confidence of the young in their scientific knowledge and ability." Applications for next year's show are already pouring in.

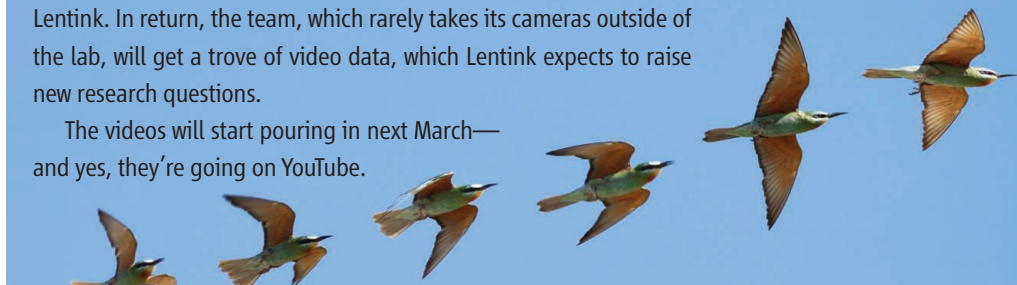
TAKING FLIGHT

Everybody knows those super-slow-motion videos on YouTube of an exploding balloon or a bullet shattering a glass of wine. But researchers in the Netherlands think there are far more interesting things to film with high-speed cameras. They're loaning out the costly devices to volunteers willing to shoot videos of flight in nature—whether it's birds, insects, bats, or plant seeds.

The project, called Flight Artists, won a \$130,000 science outreach award in October and so far has registered 800 volunteers. After basic training, participants can borrow a 600-image-per-second Casio EX-F1 for a few days to shoot whatever flying organism takes their fancy. Those with the best videos graduate to a \$100,000 Phantom v710 camera, which snaps up to 7500 images per second in high definition. "Amateurs will get to shoot the images you normally see on Discovery Channel," says project leader David Lentink, a biomechanics researcher at Wageningen University in the Netherlands.

The idea is to share the science and beauty of flying with a broader audience, says Lentink. In return, the team, which rarely takes its cameras outside of the lab, will get a trove of video data, which Lentink expects to raise new research questions.

The videos will start pouring in next March—and yes, they're going on YouTube.



Jellyfish
invasions

1464

Milestone for
meningitis vaccine

1466



Mosquito man. After tests in a cage (left), a team led by Scott O'Neill is about to release *Wolbachia*-infected mosquitoes in two Queensland towns, Yorkeys Knob and Gordonvale (map below).

INFECTIOUS DISEASES

Australia to Test 'Mosquito Vaccine' Against Human Disease

In famous efforts setting one species against another, Australian scientists imported caterpillars to block the prickly pear—a wildly invasive cactus—in the 1920s and unleashed myxomatosis on rabbits in 1950. Now they want to take biological control to the next level. In January, entomologists will start deploying a strange bacterium called *Wolbachia pipientis* in an attempt to halt disease transmission by mosquitoes.

Their target is *Aedes aegypti*, the mosquito that transmits dengue, a human viral disease that causes crippling joint and muscle pains. Recent studies have shown that infection with *Wolbachia* makes mosquitoes resistant to the dengue virus. Now, a team led by Scott O'Neill of the University of Queensland in Brisbane wants to test whether they can spread *Wolbachia* in the wild by setting free small numbers of mosquitoes infected with the microbe. It should work, they say—and it did in cage trials—because *Wolbachia* plays weird tricks on insects' sex lives, helping it spread like wildfire.

Two towns in Queensland have been selected as the testing ground. Dengue occurs sporadically in this tropical region, but the team hopes to get approval next year for a study in Vietnam, where the disease is a much bigger problem. Thailand could come next. "We wanted to start in our own back-

yard," says O'Neill, if only to show that the plan could make it through Australia's more rigorous regulatory system.

Some studies hint that the approach could work for several other diseases, including malaria, a major killer. And the field trial follows the news a few weeks ago that a rival scheme to control dengue—using genetically sterile mosquitoes—has undergone a field test on the Caribbean island of Grand Cayman (*Science*, 19 November, p. 1030). Together, they represent an exciting acceleration for the field of mosquito-control research, says Jason Rasgon, an entomologist at Johns Hopkins University in Baltimore, Maryland. "If you had asked me 5 years ago when we would start releasing *Wolbachia*-infected mosquitoes, I might have said, '20 years from now,'" he says.

Wolbachia lives inside the cells of more

than half of all insect species, including many mosquitoes, but not the most important disease vectors. Female insects pass the bacteria on to their offspring via their eggs—but with a twist. All offspring from infected females carry the microbe, but when an uninfected female mates with an infected male, there are no viable offspring. This means that, once *Wolbachia* is in, it can spread through an insect species very fast. In the past 2 decades, for instance, scientists have watched it race through *Drosophila simulans*, a fruit fly species, around the world.

Initially, scientists had different plans to use this uncanny trait. Their idea was to splice one or more genes into *Wolbachia* that would help make mosquitoes unable to transmit human disease. By setting these transgenic microbes loose in the mosquito population, they hoped to make the genes spread rapidly. *Wolbachia* would, in other words, be a vehicle.

But in an explosion of papers published in the past 2 years, O'Neill and others have shown that infection by *Wolbachia* alone makes *Ae. aegypti* resistant to dengue. No extra genes are needed; once a vehicle, *Wolbachia* has now become the weapon itself. The big advantage, O'Neill says, is that because no genetic engineering is involved, the microbes face lower regulatory hurdles and fewer angry environmentalists. "You can think of it as a vaccine for mosquitoes," he adds.

To many researchers' surprise, that same "vaccine" also appears to protect mosquitoes from chikungunya, another human virus, and from filarial worms, which cause lymphatic filariasis (LF), a mosquito-borne tropical disease better known as elephantiasis that causes





Genetic tests
versus illegal
fishing

1468



Biblical archaeology
goes high-tech

1472

grotesquely swollen limbs. *Wolbachia* even appears to inhibit several species of *Plasmodium*, the parasites that cause malaria. Just how *Wolbachia* keeps so many agents at bay isn't clear. Some studies suggest it primes mosquitoes' innate immune system to better resist invaders; others say the pathogens lose in the competition with *Wolbachia* for certain key cellular components, such as fatty acids.

Whatever the mechanism, scientists plan to exploit it to the hilt. Steven Sinkins of the University of Oxford in the United Kingdom hopes to enlist *Wolbachia* to help rid Polynesia of LF. He and Rasgon are also both hoping to develop a *Wolbachia* strain that will stably infect *Anopheles gambiae*, the most important malaria vector in the world. (So far, they have infected individ-

ual mosquitoes, but these didn't pass the microbe on to the next generation.)

For the impending dengue trial in Queensland, O'Neill's team plans to release about 10 mosquitoes a week per household for 12 weeks. The goal is to find out how well the microbe spreads. Based on mathematical models, O'Neill thinks the infection could travel about 10 kilometers a year. Not every *Ae. aegypti* mosquito in the world will eventually become infected, he says. Geographical barriers keep separate populations apart.

Its tendency to spread on its own makes *Wolbachia* an "elegant" and potentially cheap solution, says Willem Takken of Wageningen University in the Netherlands. But a release will also irreversibly change a mosquito population, he says, which is not something to be

undertaken lightly. In theory, *Wolbachia* could make the mosquitoes more prone to carry certain diseases or alter their biting behavior. But an independent analysis by Australia's Commonwealth Scientific and Industrial Research Organisation has concluded that there is a "negligible risk" that infected mosquitoes would cause harm. The study has received a green light from the Australian Pesticides and Veterinary Medicines Authority.

There is, of course, the possibility that mosquitoes or the pathogens will adapt to elude *Wolbachia*'s effects. With some luck, that will take at least a couple of decades, says O'Neill, during which time humans may have developed vaccines or another way to thwart their enemies.

—MARTIN ENSERINK

EDUCATIONAL ASSESSMENT

Shanghai Students Lead Global Results on PISA

A group of teenagers from Shanghai, China, have posted the top scores on the latest version of an international test of practical knowledge in reading, mathematics, and science. It's the first time that students from mainland China have participated in the Program for International Student Assessment (PISA), which compares the performance of 15-year-olds from 60 nations and half a dozen so-called regional economies.

The scores from Shanghai, one of China's 22 provinces, far surpass those of students from the top nations on previous PISA assessments, administered every 3 years since 2000. Their tallies of 600 in mathematics, 575 in science, and 556 in reading gave them a 38-point margin over Singapore in math, a 21-point margin over Finland in science, and a 17-point margin over Korea in reading (see tables). Singapore, which also participated in PISA for the first time, ranked in the top five in all three subjects. The test is administered by the Organisation for Economic Co-Operation and Development, which lists 500 as the average score for the 34 OECD member countries. Twelve mainland Chinese provinces participated this year, but Shanghai's are the only results that have been released.

The 2009 PISA results show that the United States continues to trail much of

the industrialized world and rising economies in Asia. The 5233 U.S. students from 165 schools, chosen to be a representative sample, did worst in mathematics. Their score of 487 puts them in a tie for 31st place, well below the OECD average of 496. In science, U.S. students occupied 23rd place, with a score of 502 that matches the OECD average of 501. Reading was their best subject, tying for 15th with a score of 500 that is statistically identical to the OECD average of 493.

The Shanghai students not only outperformed the rest of the world but also had the largest percentage of test takers performing at the highest level. In mathematics, for example, 27% of them reached Level 6, defined as capable of advanced mathematical thinking and reasoning, versus 3% of the students from OECD countries and 2% of U.S. students.

Stuart Kerachsky, head of the U.S. National Center for Education Statistics in Washington, D.C., calls Shanghai "an educational mecca" that is also much wealthier than the rest of the country. Andreas Schleicher, head of analysis for OECD's education directorate, acknowledges that "Shanghai is not representative of China" but notes that the sample does include a large immigrant population.

One silver lining for the United States was a jump in its science scores, thanks to

PISA Rankings

SCIENCE		MATHEMATICS	
Shanghai, China	575	Shanghai, China	600
Finland	554	Singapore	562
Hong Kong, China	549	Hong Kong, China	555
Singapore	542	Korea	546
Japan	539	Chinese Taipei	543
Korea	538	Finland	541
New Zealand	532	Switzerland	534
Canada	529	Liechtenstein	536
Estonia	528	Japan	529
Australia	527	Canada	527
Other notable countries		Other notable countries	
Germany	520	Australia	514
United Kingdom	514	Germany	513
United States	502	France	497
France	498	Sweden	494
Sweden	495	United Kingdom	492
Italy	489	United States	487
Russian Federation	478	Italy	483
Israel	455	Russian Federation	468
Mexico	416	Mexico	419
Brazil	405	Brazil	386

How they did. PISA ranks countries based on whether their scores are statistically significant above, on a par with, or below the OECD average.

a better performance by its lowest-ranking students. Math scores returned to 2003 levels after a dip in 2006. The 2009 reading scores for U.S. students were not significantly different from previous tests.

—JEFFREY MERVIS

ITALIAN UNIVERSITIES

Italian Parliament Passes Controversial University Reforms

The latest attempt to reform Italy's archaic university system passed an important milestone last week when the Italian Parliament's lower house approved a proposed law aimed at eliminating nepotism in academic appointments as well as improving the quality of teaching and research. The reforms must still be given a final approval by the Italian Senate, however, and it is possible, although unlikely, that the bill will be abandoned following a no-confidence vote on the center-right government of Silvio Berlusconi on 14 December.

The reforms have been contested by much of Italy's university community. Over the past few weeks, students and researchers have mounted protests up and down the country, ones that have seen university departments occupied, famous landmarks scaled by protesters, traffic blocked, and clashes with police on the streets of Rome and elsewhere. The protesters say the reforms will cut universities' already very limited funding, deprive researchers of promotion, and reduce academics' autonomy. But even among the detractors there are

many who welcome a part of the reform that would set up a new system to distribute research funding according to international standards of peer review.

Many acknowledge that Italy's university system is badly in need of an overhaul. Promotions and funding are often awarded on the basis of connections rather than merit, providing mediocre and unproductive professors with jobs for life while pushing many of the country's brightest minds abroad. The reforms, some 2 years in the making, were drawn up by Education and Research Minister Mariastella Gelmini and would set up nationwide standards for academic recruitment, transfer some governance powers from academics to administrators, and trim the huge number of courses that universities teach. Enrico Decleva, rector of the University of Milan and president of the Italian rectors' conference, says that it "presents solutions that should improve universities."

One of the popular reforms proposes that the roughly €800 million in research grants allocated each year by the ministries of education and health should instead be handed

out by panels of independent experts, 30% of whom would be non-Italians. Ignazio Marino, an opposition senator who added this to Gelmini's plans as an amendment, says that this brings Italy into line with most developed countries, adding that it is a vital step if research funding is to be allocated on the basis of merit and not as a favor to a friend. Yet most funding for Italy's researchers will still come in the form of block grants from the government, and these allocations rarely go through a peer-review process.

Critics of the overall university reforms are unhappy about a lack of new money in the plans; with universities facing a budget shortfall of about €300 million in 2011. There has also been opposition to the plan for a new tenure-track process in which, after a maximum of 8 years, a postdoctoral researcher must either gain promotion to associate professor or leave the university where they have been doing research. The government says the change should limit a university's ability to simply appoint whomever they like, because it will impose minimum quality requirements on promotion

NATIONAL INSTITUTES OF HEALTH

A Government Niche for Translational Medicine and Drug Development

National Institutes of Health (NIH) Director Francis Collins has touched a nerve in the biomedical community with a plan to create a new center focused on translational science—his biggest initiative yet. Collins said this week that the center could be up and running a year from now, despite concerns about its cost, mission, and the fact that it could mean dismantling another NIH center.

Debate over the proposal flared at an NIH advisory board meeting on 7 December. Nearly 20 groups and investigators supported by the National Center for Research Resources (NCRR), which would be partly absorbed by the new center, expressed concern that existing NCRR programs might be lost. "It's a very large organization being done on a very fast time scale, and the community that will be affected needs more time to provide input," said biochemist Mark Lively of Wake Forest University School of Medicine in Winston-Salem, North Carolina, a member of

NCRR's advisory council, before the meeting. Despite such concerns, the board voted 12-1 to create the new center.

Only Jeremy Berg, director of the National Institute of General Medical Sciences, voted no; he is "concerned that the implications for the rest of NIH hadn't been adequately discussed," he said afterward.

The proposal for the new center came from the Scientific Management Review Board (SMRB), a panel of outside scientists and NIH institute directors whose task is to find ways to streamline NIH's structure. An SMRB working group on translational medicine and therapeutics headed by Arthur Rubenstein, dean of the University of Pennsylvania School of Medicine, began discussions in May and concluded in a draft summary in November that NIH needs to do more work in this area, particularly as drug companies are cutting back on research and development.

The center would house several exist-



ing programs at NIH, including the Molecular Libraries screening program, an effort to develop drugs for rare and neglected diseases, and NCRR's Clinical and Translational Science Awards, big grants averaging about \$9 million a year that support clinical research at academic medical centers. The center would also fold in the Cures Acceleration Network, a drug-development program created by the health care reform bill but not yet funded. It would have strong ties, not yet defined, to



Political science. Opponents to Italian university reform, like these students in Rome, fear a reduction in funds and autonomy.

candidates. But existing permanent researchers say the new system is unfair because it will place little weight on experience.

Physicist Giovanni Amelino-Camelia of the University of Rome "La Sapienza" also believes that the new tenure track could stifle original research because postdocs will be tempted to follow the research interests of the professors who would promote them rather than what is most scientifically interesting. "In the U.S. or the U.K., the possibility of promotion enhances the quality of research, but here it will have the opposite effect," he predicts.

Assuming the reform plan is approved by the Senate in the coming weeks as expected, it would still face further hurdles. According to Renzo Rubele, an Italian physicist at the Free University of Brussels in Belgium, lawmakers would still need to work out a vast number of sublaws and bylaws, a process that could last up to 2 years. He says that a previous attempt at university reform in 2005, by then-research minister Letizia Moratti, came unstuck when this work was not completed.

—EDWIN CARTLIDGE

Edwin Cartlidge is a freelance writer based in Rome.

NIH's intramural Clinical Center, which could be opened to outside scientists.

The plan has raised concerns that money to launch the new center might have to come from the budgets of other institutes. Some industry and academic scientists have also questioned whether NIH can do better than companies at developing drugs. However, Steven Paul, until recently head of drug development at Eli Lilly, says his impression is that NIH will do things "that are catalytic and complementary to industry," which he calls "potentially pretty valuable."

The most controversial issue seems to be the fate of NCRR programs. The CTSA grants cost \$490 million a year, about 40% of NCRR's \$1.2 billion budget. It's not clear what would happen to the rest of NCRR's portfolio, which includes support for construction and instrumentation, primate centers, and grants for states that don't have much NIH funding. Only some would fit easily into the new center. NCRR Director Barbara Alving made a plea to expand NCRR into the new translational center and spend more time figuring out "what does industry want from NIH?"

But Rubenstein said his group felt a new organization was needed. A working group

headed by Lawrence Tabak, NIH's deputy director, and Alan Guttmacher, director of the National Institute of Child Health and Human Development, will report back in 3 months on NCRR programs. "We want to protect the programs and the people," Collins said.

NIH staffers worried that Collins had to dissolve an institute if he wanted to move quickly to establish a new one. A 2006 law that created SMRB also capped the total number of NIH institutes and centers at the current 27. Collins decided last month to combine the National Institute on Drug Abuse and the National Institute on Alcohol Abuse and Alcoholism in a new addictions institute, which would free up a slot (*Science*, 26 November, p. 1166). But that complex merger is on a longer timeline. Collins says Congress might allow NIH to preserve NCRR and exceed the cap as "sort of an overlap" for 1 year.

These proposed changes require congressional notification. Collins must send recommendations to Health and Human Services Secretary Kathleen Sebelius, who forwards them to legislators. Congress will have 180 days to object; otherwise, the new institutes will move forward. —JOCELYN KAISER

ScienceInsider

From the *Science* Policy Blog



NIH is defending its **cancer scientists whose travel** has been questioned. Senator Charles Grassley (R-IA) had complained that 16 NCI intramural scientists took 10 or more trips a year in 2008 or 2009 that sometimes cost over \$10,000, sponsored by scientific societies or companies. "It is vital that NIH scientists participate in scientific meetings," NIH brass said in a letter. http://scim.ag/nih_letter

The British charity Sense About Science has released initial polling data on how frequently medical and scientific **journal editors are threatened with libel**. A third of a small sample of medical and scientific editors reports that their journal has been threatened with libel action. The government is drafting a bill to reform U.K. libel laws. http://scim.ag/libel_poll

An Irish court has ruled that the **punishment for a biologist at University College Cork** who read aloud a scientific paper about fruit bat fellatio was "grossly disproportionate." Dylan Evans was ordered by the school to attend 2 years of sexual harassment counseling. But the judge said an admonishment or verbal warning would have been more suitable for Evans, who says, "I won my battle." http://scim.ag/bat_ruling

The president of the Kentucky Paleontological Society has criticized the state's involvement with plans by a company to build Ark Encounter, a **\$150 million biblical amusement park** related to Noah's Ark that will include models of dinosaurs. Daniel Phelps says the project, which may be eligible for up to \$37.5 million in tax breaks if built, amounts to "government entanglement with religion" and could make the state unattractive to scientists and technically minded individuals. http://scim.ag/ark_park

The new Google Earth Engine, funded by the company's philanthropic arm, provides easy-to-monitor and configure satellite data that could help poorer nations start to **monitor and protect their own forests**. http://scim.ag/earth_engine

For more science policy news, visit <http://news.sciencemag.org/scienceinsider>.



MARINE ECOLOGY

Chinese Initiative Aims to Comprehend And Combat a Slimy Foe

QINGDAO, CHINA—Physical oceanographer Wei Hao first encountered her nemesis in the summer of 2001, during a research cruise through a Yellow Sea brimming with slimy, gelatinous masses. “Jellyfish were everywhere,” recalls Wei, dean of marine science and engineering at Tianjin University of Science and Technology. Since then, three more massive blooms have beset the region. The outbreaks have Wei and other scientists worried that the Yellow Sea is on the brink of regime change, in which jellyfish supplant fish as the dominant open-ocean species. “For so long we were focused on tracking fish,” says marine ecologist Sun Song, director of the Institute of Oceanology of the Chinese Academy of Sciences here. “But now we realize jellyfish may be more important ... as a major indicator of environmental change.”

Last month, researchers met in this port city to plot strategy on a 5-year, \$4 million mission to understand the disturbing ascendancy of jellyfish in Asian waters, one that parallels the apparent rise of the animals in the Mediterranean and Caspian seas and other bodies of water (*Science*, 16 September 2005, p. 1805). “It’s

an amazing amount of money directed at a neglected problem,” says Anthony Richardson, a marine ecologist at CSIRO Marine and Atmospheric Research in Cleveland, Australia. One objective of Sun’s team of 70 researchers from six institutions is to probe the creature’s life cycle and interactions with other organisms. Another aim is to pin down the cause of blooms; in Asia, chief suspects are overfishing, climate change, and eutrophication, or excess nutrients in coastal waters. Such knowledge may prove critical to thwarting an ecological hijacking. “Once the ecosystem shifts to jellyfish, it may be impossible to go back,” warns Yu Zhigang, a marine chemist here at Ocean University of China.



Antitakeover bid. Sun Song leads the China Jellyfish Project.

Jellyfish already take a heavy toll on society. Stings kill dozens of people a year—far more than shark attacks. “It’s the most dangerous marine creature in the world,” says Sun. The box jellyfish is especially lethal: A victim can die within 3 minutes of being stung. The jellyfish’s long rap sheet also includes fouling fishing nets, killing farmed fish, closing down popular beaches, and clogging cooling intakes at coastal power plants.



Slippery as she goes. Chinese researchers haul jellyfish from the Yellow Sea last year; the Nomura’s jellyfish (left) can tip the scales at 200 kilograms.

Although data are sparse, anecdotal reports indicate that jellyfish blooms are becoming more frequent worldwide. East Asia has been particularly hard hit. In the Sea of Japan, outbreaks of Nomura’s jellyfish (*Nemopilema nomurai*), which can reach up to 2 meters in diameter and weigh 200 kilograms, once were a rare phenomenon. But massive blooms have occurred almost every summer since 2002, inflicting billions of dollars’ worth of damage to fisheries, says Shin-ichi Uye, a marine ecologist at Hiroshima University. He estimates that in 2005, up to 20 billion of the refrigerator-sized jellyfish choked the Sea of Japan. That breathtaking abundance may sound like a bonanza for researchers, but studying jellyfish isn’t easy. Even titans like Nomura’s are fragile and break easily, and fishers are loath to handle them. Satellite imaging is useless: During their medusa stage, jellyfish mostly hover meters below the surface. “We’re dealing with something that’s very hard to measure,” says Richardson.

Much about jellyfish consequently remains an enigma. One aim of the new initiative, called the China Jellyfish Project, is to shed light on the animal’s “mysterious and complicated life history,” Sun says. The vast majority of jellyfish belong to the phylum Cnidaria, which includes the Portuguese man-of-war and the three species—Nomura’s, *Aurelia aurita*, and *Cyanea nozakii*—plaguing Chinese waters. Cnidaria species can spend years on the sea bottom as polyps. These reproduce asexually, popping off medusae that drift up the water column toward the surface. In the Chinese project, scientists will search for the elusive polyps in the Yellow Sea and East China Sea. The team also plans to integrate sonar fish finders with cameras to hunt for medusae. That approach will be “much better” than using plankton nets or trawl nets, says Uye.

CREDITS: (TOP, LEFT TO RIGHT) SHIN-ICHI UYE; COURTESY OF SUN SONG; (BOTTOM) R. STONE/SCIENCE

Downloaded from www.sciencemag.org on December 9, 2010

The most pressing task may be to unravel the cause of blooms. Overfishing of certain species “opens up ecological space for jellyfish,” says Richardson. Decimated sardine stocks may be to blame for the rise of *Chrysaora* jellyfish off Namibia. Fish prey on jellyfish, so overfishing removes a check on jellyfish population growth. And jellyfish prey on fish eggs and larvae, making it harder for battered fish stocks to mount a comeback.

Another concern is eutrophication. A surfeit of nutrients from agricultural runoff and sewage spurs phytoplankton blooms in coastal waters that can trigger jellyfish outbreaks. Such conditions also create low-

oxygen dead zones. Compared with fish, jellyfish—both polyps and medusae—are much more tolerant of hypoxic conditions. Because the number of marine dead zones worldwide has doubled each decade since the 1960s, there’s more habitat better suited to jellyfish, says Richardson.

Recent climate change also appears to have given jellyfish a helping hand. Warmer ocean temperatures are correlated with jellyfish outbreaks, “but we don’t know the mechanism,” says Sun. He and others fear that warming, overfishing, and eutrophication may work in concert to create jellyfish-dominated ecosystems reminiscent of those during the Precambrian world, more

than 550 million years ago.

The waters off China may be nearing the tipping point beyond which fish predators are unable to hold jellyfish in check. In the past 5 years, says Sun, anchovy catches in the Yellow Sea have decreased 20-fold. During a Yellow Sea research cruise in the summer of 2009, jellyfish constituted 95% of hauls. In coordination with colleagues in Japan, Korea, and Russia, Sun says, field surveys and modeling may allow researchers to calculate “how many jellyfish it will take to change the ecosystem.” That, in turn, could lay the groundwork for interventions that forestall doomsday for fish.

—RICHARD STONE

CLIMATE CHANGE

El Niño Lends More Confidence to Strong Global Warming

One of the biggest technical uncertainties in gauging how hot it will get by century’s end lies with clouds. How will they react to global warming? Two scientists have independently argued from observations that warming will alter clouds in ways that will largely counter warming by greenhouse gases. But computer climate models show clouds changing in ways that amplify warming, not dampen it. The overwhelming majority of climate scientists sides with the models. Whom to believe?

To help sort it out, climate researcher Andrew Dessler of Texas A&M University in College Station looked at the example of El Niño and La Niña, naturally occurring weather patterns that cause warming (El Niño) and cooling (La Niña) in the tropical Pacific and around the globe. In a report on page 1523, he analyzes how they have actually influenced clouds. His result is “convincing evidence” that—at least on the scale of decades—clouds do not counter warming, says climate researcher Brian Soden of the University of Miami in Florida. Many of Soden’s climate colleagues would agree, but even Dessler notes that “my analysis doesn’t settle the question” of just how much the world can warm.

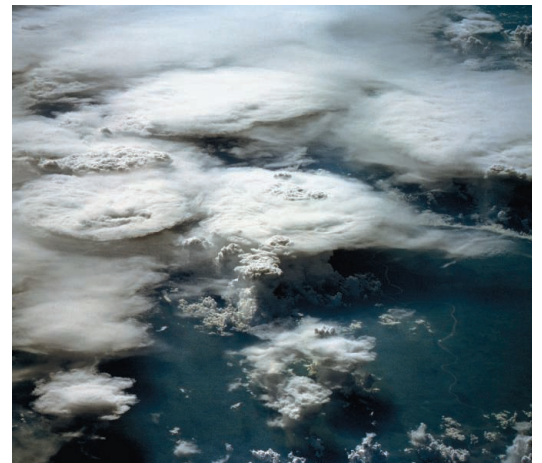
As others have done before, Dessler used the global warmings and coolings induced by El Niño and La Niña every few years as a substitute for the long-term warming to come. In a warmer greenhouse world, the high, wispy clouds that tend to trap heat that would otherwise escape to space might thicken or expand to cover larger areas. Such a positive feedback would warm the planet further. Or greenhouse warming might thicken or expand low-lying clouds that tend to reflect solar energy to

space, cooling the world. That would be a negative feedback.

In principle, scientists could tell whether negative or positive feedbacks prevail under a warming by comparing observed surface temperature with the amount of energy actually radiating to space, but satellite records of such radiative losses are too short to reveal what has happened in the past century. So researchers have had to rely on records of only a decade or two. Dessler did the same but with some differences. He considered the whole globe, not just the tropics or single regions as previous researchers had done. He used only the past 10 years of satellite data, the best observations available. And he removed feedbacks due to other factors, such as changing water vapor.

Dessler found a small positive feedback overall. He writes that, given the scatter in the data, he “cannot exclude the possibility of a small negative feedback,” but he sees “no evidence to support ... a large negative cloud feedback” capable of counteracting global warming. When Dessler analyzed the cloud feedbacks in eight leading global climate models in the same way, he found that on average the models produce a similarly small positive feedback during a century. That provides “some indication that models successfully simulate the response of clouds to climate variations,” he writes.

“This is a very important check of the models,” says climate researcher Qiang Fu of the University of Washington, Seattle. “It shows no evidence of a large negative



Whither clouds? As the greenhouse strengthens, changing clouds may either amplify or minimize the warming. A new analysis shows no sign that the warming will be countered.

cloud feedback.” But climate researcher Roy Spencer of the University of Alabama, Huntsville, disagrees. He published one of the two papers finding evidence of a strongly negative cloud feedback. He finds in his own analyses signs that Dessler is seeing not only cloud changes caused by temperature changes but also temperature changes caused by natural cloud fluctuations. Such effects garble the true negative feedback beyond recognition, he says.

Spencer’s “interpretation is wrong,” says Soden, but even if Spencer were right that there’s a cause-and-effect problem, Dessler’s method of comparing observations and models “eliminates some possibilities, such as the models being egregiously wrong. It’s about as good as we can do with current data sets.”

—RICHARD A. KERR

VACCINE INTRODUCTION

The Beginning of the End for Africa's Devastating Meningitis Outbreaks?

Nine years ago, a small group of infectious-disease experts gambled on an unorthodox strategy to make a much-needed—and affordable—vaccine for Africa. Last Monday in Burkina Faso, it paid off in spades with the kickoff of a massive campaign to immunize 20 million people in three African countries against deadly meningococcal meningitis by the end of December. The eventual goal, if the money comes through, is to immunize some 250 million people in 25 countries over the next several years, putting an end to the ferocious epidemics that regularly sweep across Africa's so-called meningitis belt (*Science*, 27 June 2008, p. 1710).

MenAfriVac, as it is called, is the first new vaccine made to the highest interna-

All-star cast. Burkina Faso's first lady, Chantal Compaoré, was one of the dignitaries at the launch of a new meningitis vaccine, which promises to put an end to the deadly epidemics in Africa's meningitis belt (above).



tional standards and designed specifically for Africa—and for a particularly African scourge, meningitis A. (Meningitis A is virtually nonexistent in Western countries.) What's most remarkable, experts say, is its price, a mere 44 cents a dose, and the speed with which it was developed and delivered—less than 10 years from when the idea was hatched.

"That quick an introduction hasn't happened before," says Chris Elias, president of the nonprofit PATH in Seattle, Washington, which has played a key role in developing this

and other vaccines for poor countries. "I think it is one of our most important milestones of the decade. And I'm not the only one who thinks so," Elias said in an interview from Abuja, Nigeria, en route to Burkina Faso to witness the symbolic first shot.

Also there to celebrate were Blaise Compaoré, the president of Burkina Faso; Margaret Chan, director-general of the World Health Organization (WHO); Tachi Yamada, head of the Global Health Program at the Bill and Melinda Gates Foundation, who calls MenAfriVac an "amazing success"; and Helen Evans, interim CEO of the GAVI Alliance. And, of course, Marc LaForce, who

shepherded the vaccine through its rocky birth and adolescence as head of the Meningitis Vaccine Project, a collaboration of PATH and WHO that was launched with a large grant from the Gates Foundation. "I'm pretty excited," says the 71-year-old LaForce, who says witnessing the launch was "humbling."

Until now, there has been no way to prevent the deadly meningitis epidemics that erupt across a swath of sub-Saharan Africa (see map) each year with the onset of the dry season in January or February and stop abruptly with the rains in May or June. (Burkina Faso, Mali, and Niger, the three countries where the vaccine is first being launched, are particularly hard hit.) An infection of the thin membrane that lines the brain, meningococcal meningitis can kill within 24 hours and often leaves its survivors deaf or intellectually impaired. Several strains of *Neisseria meningitidis* can cause meningitis, but group A accounts for about 85% of epidemic disease.

An older polysaccharide vaccine was

developed in the 1960s. But its effectiveness is so limited—immunity lasts for just 2 or 3 years, and it has minimal benefit in children under 2—that it is used only as a "Band-Aid" in reactive campaigns to limit the spread of epidemics that have already begun. The vaccine often arrives too late to do much good.

The new conjugate vaccine uses the same polysaccharide from the bacterium's coat but links, or conjugates, it to a protein that makes it far more immunogenic. LaForce thinks that MenAfriVac will protect for at least 10 years. And unlike the older vaccine, the conjugate prevents those infected from transmitting the bacteria, thus conferring "herd immunity," or protecting those who don't receive the vaccine. It is modeled on a far more expensive conjugate vaccine that has all but wiped out the related meningitis C in several European countries.

When LaForce took on the job, he quickly realized that the accepted strategy—working with big pharma to adapt a vaccine, then negotiating to get the price down—would not deliver a vaccine that met his definition of "affordable." A conjugate pneumococcal vaccine now poised for introduction in developing countries, for instance, costs roughly \$3.50 a dose. Instead, LaForce insisted on a guaranteed selling price of less than 50 cents and set out to find partners who could make it happen. When big pharma did not step up, LaForce hatched a scheme to work with an Indian manufacturer, Serum Institute of India Limited, among others. Some of his partners in the project were skeptical, says LaForce.

"We asked some tough questions," recalls Elias. "Marc was convinced it was going to work. But he had to convince the global health community." It was risky, he says, to "work with a company that did not have a major research focus like big pharma and that hadn't worked with a conjugate vaccine before, in a country that had never regulated a conjugate vaccine before."

Now Elias calls MenAfriVac a model for delivering all sorts of public health interventions, not just vaccines, to poor countries. So does Yamada, who says the Gates Foundation is investing in a new pneumococcal vaccine that is being developed at Serum in India as well. "We have high hopes it will come in well under \$3.50," says Yamada.

But despite all the fanfare surrounding last week's launch, LaForce notes that MenAfriVac's widespread introduction is by no means assured. In 2008, GAVI approved the \$370 million plan for the full vaccine rollout, but, in an unexpected move, the financially strapped organization provided limited funding—\$29.5 million—for intro-



duction in just the first three countries, as well as \$55 million to stockpile the old vaccine for emergencies until the new one is widely available. “It’s an ongoing challenge, what will GAVI do for the next countries,” says LaForce, who says he won’t breathe easily until the money is in the bank.

Helen Evans of GAVI says the organization, facing a roughly \$4 billion funding gap over the next 5 years, is committed “in prin-

ciple” to providing the full \$370 million by 2015—if GAVI’s donors step up. Otherwise, “choices will have to be made about which vaccines to prioritize,” adds a spokesperson.

The vaccine must still prove its mettle in the follow-on studies that are already beginning. A key issue is serotype shifting. The worst case, which LaForce considers unlikely, is that the vaccine will prove effective against type A, but then another strain would move in

to fill that niche. That’s why work is already beginning on a multivalent vaccine, says Elias, but it will be harder to make and will undoubtedly cost more.

In the best case, Elias says, if the meningitis A vaccine works as expected, those districts that were immunized in December will be spared this season’s epidemic. “We should know by June,” Elias says.

—LESLIE ROBERTS

ScienceNOW

From *Science’s* Online Daily News Site

Why Diets Fail

Bad news for dieters: New research shows that dieting makes the brain more sensitive to stress and the rewards of high-fat, high-calorie treats. And that can cause you to regain lost weight.

Stress triggers the release of cortisol, a “fight or flight” hormone that, if chronically elevated, can cause increased appetite and weight gain. Tracy Bale, a neuroscientist at the University of Pennsylvania, and colleagues hypothesized that dieting leaves people more susceptible to the stresses of everyday life, leading them to pack on lost pounds.

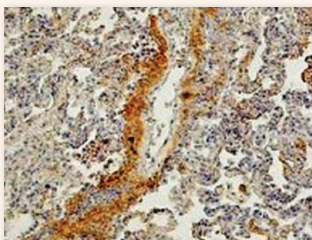
To test the idea, the team put lab mice on a 3-week diet until the rodents had lost about 10% to 15% of their original body weight. After exposure to mild forms of stress, the mice’s blood cortisol levels shot up higher and stayed elevated longer compared with levels in control mice. But even after a week of normal eating, the ex-dieters remained more sensitive to stress and were more likely to eat large amounts of high-fat mouse chow when under pressure, the team reports in *The Journal of Neuroscience*.

The team also found that the diet, although short, resulted in long-term changes in gene expression that would undermine any dieter’s efforts. The mice that dieted had significantly higher levels of the protein that stimulates cortisol release, indicating higher sensitivity to stress, as well as higher levels of appetite-stimulating hormones after exposure to the high-fat binge food. This may help explain why so many diets fail: Dieting increases stress sensitivity, and stress makes us seek relief in high-fat, high-calorie “comfort” foods. <http://scim.ag/diet-fail>

How Swine Flu Killed the Healthy

One of the most baffling questions of the 2009 H1N1 “swine flu” pandemic was why the virus killed healthy individuals while sparing the very young and the very old. The answer may be an immune system gone haywire, according to new research.

Fernando Polack of Vanderbilt University in Nashville and his colleagues found that lung samples from 75 young and middle-aged adult victims of the 2009 pandemic contained a surprising amount of a protein called C4d. C4d usually binds to antibodies to form virus-fighting immune complexes. But in this case, he says, C4d probably did more harm than good. That’s because the adult antibodies—trained to fight sea-



The lungs of this adult H1N1 victim contain C4d (orange).



Pollutant Changes Sexual Preference

A new study shows that mercury in the environment can change an animal’s mating habits. Peter Frederick of the University of Florida, Gainesville, and Nilmini Jayasena of the University of Peradeniya in Sri Lanka raised 120 wild white ibis chicks for 3 years, lacing some of the birds’ diets with mercury. The team reports in *The Proceedings of the Royal Society B* that 55% of male birds in the group exposed to the highest level of mercury, 0.3 parts per million, formed mating pairs with other males. Male-male pairs can occur in the wild when females are unavailable, but these birds had an ample supply of mates. Although the mechanism is unknown, the study suggests that mercury, a common pollutant from coal-burning power plants, may threaten the survival of ibises and other species, as no laws exist to protect them from exposure. <http://scim.ag/mercury-changes>

sonal flu—were a poor match for H1N1. They recognized the virus and latched on to it but weren’t able to stop it from replicating, says Polack.

Unable to fight back, the system spiraled out of control, Polack speculates. Instead of punching holes in the viruses, the C4d-antibody complexes punctured the victims’ veins, flooding their lungs with water and plasma, the team reports in *Nature Medicine*.

It makes sense that the old and the young didn’t have this strange reaction, Polack says. Young children and infants have few or no antibodies against seasonal flu strains, whereas elderly people had antibodies to an earlier H1N1 strain, known to be a much better match for the 2009 version. <http://scim.ag/H1N1-victims>

Read the full postings, comments, and more at <http://news.sciencemag.org/sciencenow>.



To Fight Illegal Fishing, Forensic DNA Gets Local

A new generation of genetic tests could give authorities a much better idea of exactly where fish have been caught

IN 2003, GENETICIST EINAR NIELSEN OF the Technical University of Denmark got an unexpected phone call from Danish fisheries inspectors. They suspected that a fishing vessel had violated its quota by catching too many cod from the North Sea. But the captain claimed that he had caught the fish legally in the Baltic Sea. The difficulty for the authorities was that the fish from both places were the same species, the Atlantic cod (*Gadus morhua*), and they look alike. Nielsen had been studying the genetics of the Atlantic cod, however, and he used DNA markers called microsatellites to show that the fish in question were very likely from the North Sea. A judge agreed, fining the captain \$8800 and confiscating his \$44,000 catch.

It was a rare victory against the massive problem of illegal fishing. For technical reasons, however, microsatellite tests for identifying the local origins of caught fish haven't been widely adopted. A €3.9 million European research project, called FishPopTrace, aims to now lay the groundwork for a different kind of test that could be broadly useful not only for enforcing fisheries regulations but also for catching fraudulent labeling of

fish in supermarkets.

The consortium, which began in 2008, is exploring a range of possible techniques, such as protein patterns in fish tissue and the composition and shape of ear bones called otoliths. But the group is betting heavily on genetic variants called single-nucleotide polymorphisms (SNPs). That's because SNPs should lead to tests that are faster, cheaper, and easier to scale up than those based on microsatellites. The ultimate payoff is the advent of reliable and widely used tests to determine not just which species of fish has been caught but which particular local population it came from. "Being able to take [enforcement] to the population level is a big step forward," says Michael Hirshfield, chief scientist of the advocacy group Oceana, based in Washington, D.C., who is not a member of the consortium.

At a meeting last month in Madeira, Portugal, the FishPopTrace consortium discussed its results, and although most of its work is unpublished, other fisheries experts

Illegal catch. Fisheries inspectors such as these can't nail all violators, but genetic tests may prove another deterrent.

there say the use of SNPs is promising. "This is going to explode," predicts Kevin Glover of the Institute of Marine Research in Bergen, Norway. "They've shown it's feasible." In February, the consortium will present final results to policymakers, environmental groups, and others at a meeting in Brussels.

Fishing fraud

Although it's difficult to get reliable information on the extent of illegal fishing, researchers estimate that this shadowy business is worth as much as \$23 billion worldwide each year. Sometimes the violations consist of boats catching more fish than allowed. More egregious is the taking of fish from areas that have been closed to recover from overfishing. Another problem is consumer fraud: the intentional mislabeling of fish as a more valuable species or as coming from a more desirable location.

Government agencies fight illegal fishing using a variety of tools. Some place observers on larger vessels to keep an eye on what's hauled in, checking that only fish of the right size are caught, for example. In the European Union, larger boats must have GPS units to make sure they're not fishing in off-limits areas. But observers can't follow all fishing vessels, and monitoring systems can be bypassed. So when it comes to catching scofflaws, forensic genetic approaches that distinguish between species have been critical. For more than 20 years, DNA tests have been used to identify species that have been illegally caught or mislabeled.

It is much harder to track a fish to its native population. DNA tests look for unique markers, and there are fewer genetic differences between populations of the same species than between species. This is particularly true in the oceans, where populations tend to be large and overlap, which can wash out genetic differences between locales. The microsatellite approach, used by Nielson for cod, can find markers unique to populations, but it isn't particularly efficient or always feasible. And the technique hasn't been scaled up as an enforcement tool, in part because of calibration issues between laboratories.

In December 2006, the European Commission put out a call for proposals to develop tools to better characterize populations of marine fish and improve the traceability of fish products. FishPopTrace, coordinated by Gary Carvalho of Bangor University in the United Kingdom, won the competition.

Online

sciencemag.org



Podcast interview with author Erik Stokstad.

FishPopTrace is focusing mainly on SNPs, specific nucleotides in a DNA sequence that can vary and thus can help tell apart individuals or species. SNPs are much more common in the genome than are microsatellites, which improves the odds of finding patterns specific to different populations. This approach has been successfully used by managers to distinguish among Pacific salmon, whose populations spawn in particular streams and rivers and tend to be more genetically distinct than fish that spend their whole lives in the ocean.

To see whether they could expand the technique to exclusively marine fish, Carvalho's team picked four economically valuable species as test cases: Atlantic cod, European hake, common sole, and Atlantic herring. These species represent different life habits and geographic ranges, and all suffer from illegal fishing. The researchers obtained samples from about 50 fish from each of 20 populations around European waters, then team members at Aarhus University in Denmark sequenced the samples and identified possible SNPs. By June 2009, they had created one "SNP chip" for each of three species: sole, hake, and herring. (Canadian researchers had already created a SNP chip for cod to assist in aquaculture research.) These DNA-covered microchiplike devices can test the identity of 1536 possible SNPs. For each population, they tallied up the frequencies of all the SNPs, creating what they hoped would be a diagnostic pattern.

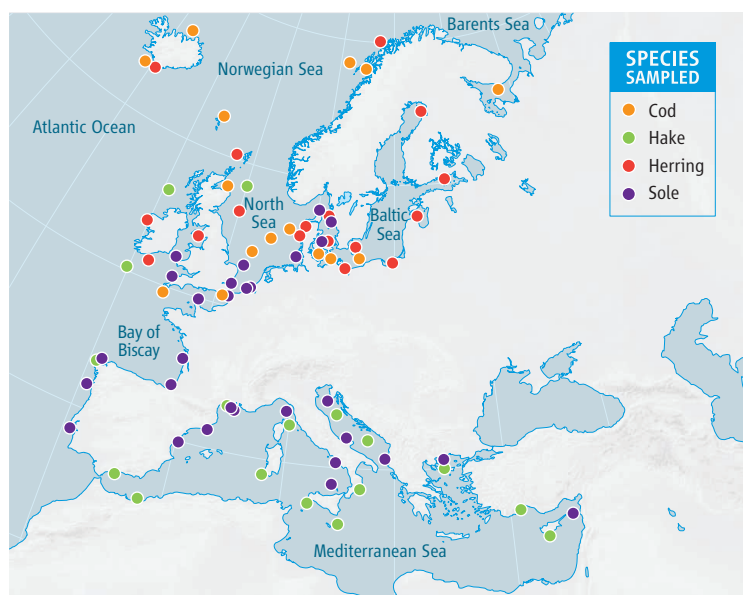
The next step was to find out if the SNP chips could accurately and reliably distinguish among samples from different populations: a North Sea cod from a Baltic one, as Nielsen had done with microsatellites, for example. An important part of this process was figuring out how few SNPs were needed; minimizing the number will lead to cheaper, simpler tests.

Fish and chips

The researchers investigated questions relevant to fisheries and to European consumers. A common concern for the latter is the source of Atlantic cod. Fish from the Baltic are worth less because they tend to have lower quality flesh and higher levels of contaminants. The cod team used its SNP chip

to examine, without knowing the source, samples from both locations. By looking at 20 SNPs, the researchers correctly identified every sample's origin. With just 10 SNPs, 96% of the samples were still correctly identified. "The results look very promising," Nielsen says.

The SNP chip for sole (*Solea solea*) also performed well. This flatfish fetches the highest price of the four species and is severely overfished in Europe. A key question is whether sole from the North Sea can be distinguished from populations in the Mediterranean, which are considered to be higher quality. Just one SNP could reveal which sole was which with 96% accuracy. "Technically, it's a piece of cake," says



Population control. Genetic analysis of fish populations from across European waters is revealing patterns that can be used to identify the source of fish.

Filip Volckaert of the Catholic University of Leuven in Belgium.

European hake (*Merluccius merluccius*) has been a contentious species because of differing regulations. Atlantic hake must be 27 centimeters long to be legally landed. But in the Mediterranean, vessels can catch hake that are only 20 cm. So fishing vessels in the Bay of Biscay sometimes catch smaller fish and then misreport their origin as the Mediterranean. The FishPopTrace team showed that just 10 SNPs could reveal the origin of hake with near-perfect accuracy.

The most demanding test case was herring. An abundant, migratory species with a wide distribution, European herring have long resisted attempts to tease apart genetically distinct populations. By looking at SNPs, however, it was possible to accurately distinguish herring, such as those

in the northeast Atlantic and those in the North Sea—a goal important to a joint E.U.-Norwegian management plan. But more SNPs were required to identify fish from various sources around the United Kingdom, where there is substantial misreporting of catches. Further refinements could reduce the number of SNPs, says Sarah Helyar, a postdoctoral researcher at Bangor University.

FishPopTrace is also checking the rate at which local fish populations evolve, a determinant of how long their SNP tests may be useful. For each species, the teams are using computer models and looking at decades of tissue samples to see if specific fish populations will maintain the same patterns of SNP frequencies over time.

Rob Ogden of TRACE Wildlife Forensics Network, a nonprofit in Edinburgh, U.K., is validating the consortium's new genetic tests. This involves confirming that they work under a range of conditions and creating standard operating procedures for other labs to use. "It's absolutely essential if you want to move from an academic environment to testing," Ogden says. And there is growing interest among regulators; a major E.U. fishing law passed last year mentions genetic tests explicitly as possible enforcement tools. "I'm pretty convinced that they will be much more extensively used," says Jann Martinsohn, who studies marine policy at the European Commission's Joint Research

Centre in Ispra, Italy.

How SNP-based tests are ultimately used—by port inspectors, for example, or commercial labs testing frozen fillets—will depend on their accuracy, cost, and ease of use. SNPs that end up on the witness stand may remain the exception, Ogden predicts. He says that just the act of random testing will encourage compliance, at a fraction of the cost of litigation.

That view matches Nielsen's courtroom experience, which was the first introduction of genetic testing of populations to the Danish fishing fleet. "The case did certainly have a deterrent effect," he says. "They know that we can test, so they are probably less likely to put themselves in situations where testing could reveal fraud." The task now is to create genetic tests that will broaden the scope of this deterrence.

—ERIK STOKSTAD

MATERIALS SCIENCE

What Shall We Do With the X-ray Laser?

A newfangled x-ray source exceeds expectations in creating new conditions of matter, probing materials, and deciphering the structures of proteins

MENLO PARK, CALIFORNIA—A year and a half ago, the SLAC National Accelerator Laboratory fired up the world's first hard x-ray laser. Shining 10 billion times as bright as any previous x-ray source, the Linac Coherent Light Source (LCLS) would probe matter in new ways: simulating conditions within a planet's core, resolving the ultrafast changes in a material's atomic-scale architecture, and determining the structure of a protein from individual molecules. Or so claimed the machine's designers. Some potential users questioned whether the \$420 million LCLS would live up to its billing.

Now scientists have results from the first experiments with the machine, which they discussed at a recent meeting here.* Although it's too soon to say that the LCLS will succeed at every task—three of six experimental “end stations” are not yet finished—the x-ray laser has already produced novel conditions of matter and demonstrated unprecedented capabilities. If anything, it appears to be more reliable, versatile, and fruitful than expected. “I think it's already been transformational,” says biophysicist Jasper van Thor of Imperial College London. Linda Young, an atomic physicist at Argonne National Laboratory in Illinois, likens using the LCLS to rocketing to the moon the first time: “Everything you do is new.”

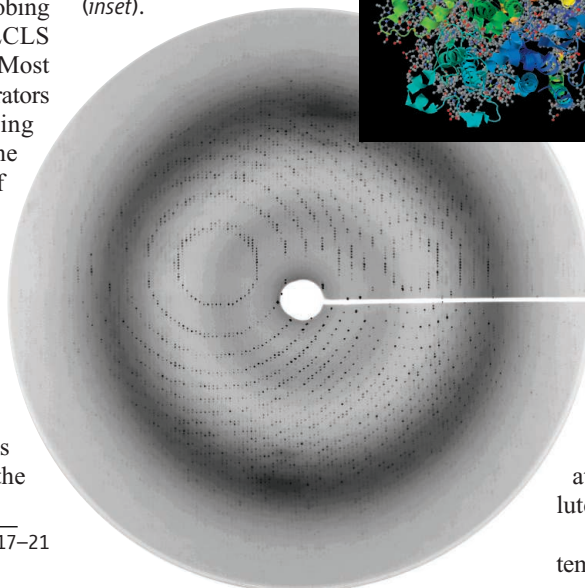
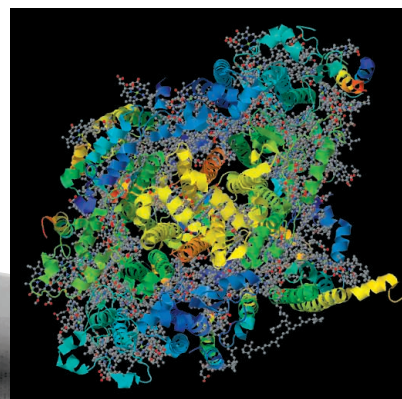
X-rays are scientists' best tool for probing matter on the atomic scale, and the LCLS is an x-ray source unlike any before. Most large sources are circular particle accelerators called synchrotrons. Electrons whizzing around them radiate x-rays, a bit in the way a twirled dish rag flicks off drops of water. In contrast, the LCLS is an x-ray free-electron laser (X-FEL) that uses a linear accelerator, or linac, to fire bunches of electrons through a chain of magnets called undulators. The undulators make the electrons wiggle and generate x-rays.

Traveling along with the electrons, the x-rays push them into subbunches that radiate far more effectively than the

individual particles. Akin to feedback in a public-address system, that effect produces a hugely intense burst of x-rays. The photons in the pulse also wiggle in synchrony, like those in ordinary laser light. Moreover, the LCLS's pulses are extremely short, lasting as little as a few million-billionths of a second, or femtoseconds. So they can take snapshots of lightning-fast atomic-scale changes.

When the LCLS turned on in April 2009, experimenters first shined the x-rays on a gas. Even that rudimentary study created something new: made-to-order “hollow” atoms. The electrons in an atom stack into “shells” with definite energies, and the innermost shell contains the two most tightly bound electrons. An x-ray with enough energy can knock out one of these electrons, although within femtoseconds, an electron from an outer shell falls into the vacancy. Rarely, the fleeing first inner-shell electron takes the second one with it, leaving a hollow atom with an empty inner shell.

In a flash. Blasting nanocrystals, biologists confirmed the diffraction pattern, ground out at a synchrotron, of the membrane protein PSI (inset).



The LCLS shines so brightly that physicists can hollow out atoms at will. Argonne's Young and colleagues shined x-rays with an energy of 2 kilo-electron volts (keV) onto xenon. Had those pulses come from a synchrotron, each atom would have been hit by at most one photon. But a 230-femtosecond LCLS pulse bombards each atom with multiple photons, enough to strip some xenon atoms of all 10 of their electrons, the team reported in the 1 July issue of *Nature*. Moreover, by making the x-ray pulse shorter and more intense, the physicists could blast out both inner-shell electrons and leave the atom hollow.

Paradoxically, such a double inner-shell vacancy persists longer than a single vacancy. It also renders an atom momentarily transparent to the x-rays, which are tuned to knock out only inner-shell electrons. So hollowing out atoms leads to a general slowdown of the electron-stripping process, which is how the scientists could tell they were making the atoms hollow in the first place.

In fact, they deduced from the yields of xenon atoms with different numbers of electrons that the shortest pulses lasted as little as 20 femtoseconds, far shorter than the aimed-for 80 femtoseconds. Accelerator physicists said the pulse couldn't be that short, Young says, “but I said, ‘I'm sorry, but that's what the measurements show.’” LCLS operators

can now make pulses a couple of femtoseconds long, too short to measure. That should make the LCLS even better at probing ultrafast atomic-scale processes.

Condensed matter physicists have also been pleasantly surprised by what the LCLS can do. Wei-Sheng Lee of Stanford University

in neighboring Palo Alto, California, and colleagues used it to track ultrafast changes in electronic and magnetic patterns within a material and found an unexpected relationship between them. They studied lanthanum strontium nickel oxide, which is a relative of lanthanum strontium copper oxide, a high-temperature superconductor that can carry electricity without resistance at temperatures as high as 40° above absolute zero.

Physicists still do not agree on how high-temperature superconductors work. The cop-

*2010 LCLS/SSRL Users' Meeting & Workshops, 17–21 October 2010.

per oxide superconductor has planes of copper and oxygen ions in a square pattern with lanthanum and strontium layers between them. In lanthanum strontium nickel oxide, nickel replaces copper. Within the superconductor, free-moving electrons sit at most one to a copper ion. At temperatures above the superconducting transition, those on alternate rows spin in opposite directions to create stripes of magnetism. At the same time, some rows lack such electrons, leaving separated stripes of charge. The stripes may compete with or promote superconductivity.

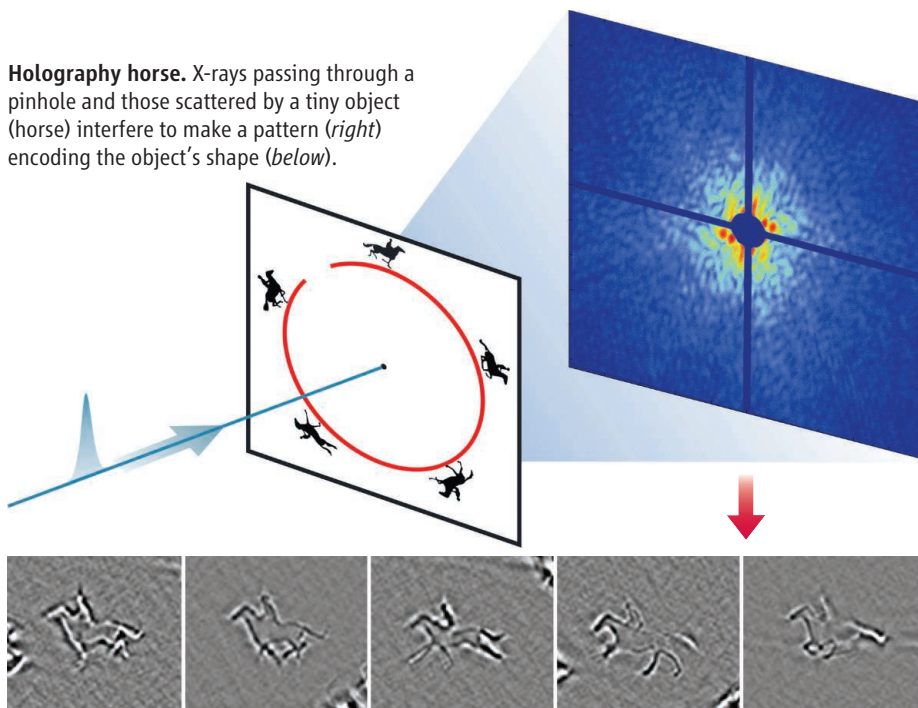
Similar stripes appear in the nickel compound, so the physicists used it to study the two types of stripes without having to account for superconductivity. They shook up their sample with a 50-femtosecond pulse from an infrared laser and, after a variable delay, applied a 70-femtosecond x-ray pulse to probe the fading and recovery of the stripes. They increased the intensity of the infrared pulse and found that the charge stripes faded completely at a lower intensity than did the spin stripes. It was “a big, big surprise” that the charge stripes vanished first, says Thomas Devereaux, a SLAC theorist not involved in the work, as it’s easier to imagine electrons arranging their spins if they’re already in rows.

As important as the result was the fact that the experiment worked at all. Physicists had worried that the LCLS’s ultraintense pulses might wipe out the stripes and obliterate the sample. However, the team attenuated the x-rays enough to preserve the stripes and still detect a signal. “We all did one of these wipe-your-forehead-in-relief things when that happened,” Devereaux says. That success suggests that the LCLS may be handier than hoped for condensed matter physics.

The most tantalizing results at the meeting may have been those probing the atomic structure of proteins. Using less-intense x-ray synchrotrons, biologists have mapped the structures of tens of thousands of proteins. They make a crystal from many copies of a protein and then scatter x-rays off the planes of atoms in it, a process called diffraction. The directions in which the x-rays emerge reveal the structure of the crystal and, hence, the protein.

That requires crystals at least a few micrometers wide, and some proteins, especially those embedded in a cell’s membrane, will not readily form crystals so large. However, the LCLS shines so brightly that it might reveal the structure of a membrane protein if researchers drop into the beam either “nanocrystals” hundreds of nanometers wide or even individual molecules. The x-rays would scatter off the tiny samples, even as they blow them apart.

Holography horse. X-rays passing through a pinhole and those scattered by a tiny object (horse) interfere to make a pattern (right) encoding the object’s shape (below).



Early results suggest that the technique works, at least for nanocrystals. A team of 84 researchers used the LCLS to study a membrane protein called photosystem one (PSI) that plays a role in photosynthesis. The researchers sprayed a jet of nanocrystals into a beam of 2-keV x-rays. They weren’t quite able to resolve the atomic structure of the protein. However, data from 3 million individual shots yielded a diffraction pattern consistent with the one produced at a synchrotron in 2001, after a decade and a half of trying to grow large enough crystals of PSI.

The result shows the LCLS’s immense potential to study hard-to-crystallize proteins, says van Thor of Imperial College, who was not involved in the work. “If you can make nanocrystals, then you can skip these 15 years and just go to the LCLS,” he says. Researchers will reach atomic resolution simply by using more energetic x-rays, and studying membrane proteins will consume much of the LCLS’s beam time, van Thor predicts.

Others are not so sure that structural biologists will flock to the LCLS. Michael Blum, an x-ray crystallographer with Rayonix, a company in Evanston, Illinois, that makes x-ray detectors, says researchers at synchrotrons are improving their work with tiny crystals and predicts biologists will take more interest in the LCLS’s ability to resolve rapid changes in proteins. It remains to be seen whether scientists can determine structures from individual molecules, he says: “Going from big crystals to small crystals is a step, but there’s a leap in going from small crystals to no crystal.”

Scientists are performing myriad other experiments, too. Some have used the LCLS

to pump energy into solid carbon, simulating conditions in the cores of planets. Exploiting the x-rays’ laser qualities, others are honing holographic techniques that might serve, for example, to trace rapid changes in magnetic materials. In the next few years, LCLS officials want to see which experiments pay off most handsomely, says Joachim Stöhr, SLAC’s associate director for the LCLS. That’s a key consideration because, unlike a synchrotron, the LCLS can service only one experiment at a time, making beam time scarce and precious. “The biggest challenge for us is to give you more beam time,” Stöhr told LCLS users. “How do we do that?”

The answer is expansion. The LCLS uses 1 kilometer of the lab’s 3-kilometer-long linac, which provided beams for particle physics experiments for 42 years, to fire electrons through one chain of undulators. By 2017, researchers plan to use a second length of linac to power two more undulator chains, with an eye to even bigger upgrades by 2025. They also hope to split the x-ray beam to run some experiments simultaneously.

That extra capacity will come in handy, as the LCLS will soon have competition. Researchers in Germany, South Korea, and Switzerland are building X-FELs that will turn on in a few years. And scientists at the SPring-8 laboratory in Harima Science Park City, Japan, will begin operating their X-FEL in March. With new linacs, those machines will produce x-ray pulses at higher rates than the LCLS. So the pressure is on researchers at SLAC to make the most of their head start.

—ADRIAN CHO



New dig. Modern tools, seen at Megiddo, uncover the past, including residues in chalices (*inset*).

A Change of Biblical Proportions Strikes Mideast Archaeology

It was the scholarly version of a military “shock and awe” campaign, quipped one archaeologist. At a packed evening session on 18 November, a series of researchers came to the podium for 2 hours of data-rich presentations—and put their colleagues on notice that their field is in the midst of a scientific revolution.

Biblical archaeology has often been heavy on textual analysis and slow to adopt scientific methods such as radiocarbon dat-

ing. Attempts to prove the accuracy of biblical accounts or to legitimize Jewish claims to the region have dogged the field. Now archaeologist Israel Finkelstein of Tel Aviv University and structural biologist Steve Weiner of the Weizmann Institute of Science in Rehovot are revolutionizing the region’s archaeology by applying a host of new technologies. Their 5-year, \$4 million effort, funded by the European Research Council, includes more than 40 collaborators from

many disciplines working at an array of sites, primarily in Israel. Finkelstein says the goal is to overcome the “strong ideological agenda” pervading the field. The team has adopted state-of-the-art methods, including analyzing human and animal DNA and ancient pollen, to resolve controversial questions about the pace and timing of migrations and construction, such as the size and power of 10th century B.C.E. Jerusalem in the time of David and Solomon (*Science*, 2 February 2007, p. 591).

Work has been under way for a year and a half, and the first results are coming in. For example, at the large Canaanite site of Tell es-Safi—possibly the home of the biblical Goliath—archaeologists long assumed that a heavy layer of ash in one area demonstrated destruction by fire in the turbulent 9th century B.C.E. That seemed to match the biblical accounts of destruction at the hands of the neighboring King of Aram. But infrared spectrometry showed that floor clays had not been heated to high temperatures, and nearby ceramics showed evidence of lipids, which would have been burned away by a fire. Although exactly what did happen is not fully clear, the ash apparently was not part of a single catastrophe but was dumped there, perhaps after a series of par-

FROM ROY KING; JOSÉ-MANUEL BENITO ÁLVAREZ/WIKIPEDIA (INSET)

Tracking the Med’s Stone Age Sailors

Remains of Neolithic settlements dot the Mediterranean’s islands and coastlines. Where did these seafaring migrants come from, or did indigenous peoples pick up technology from their neighbors as new ways of life, including farming, spread around the region?

Genetic studies are helping to fill in key pieces of the puzzle. By carefully sorting genetic data from living people, Roy King of Stanford University in Palo Alto, California, said in a talk that around 6000 B.C.E., early seafarers indeed spread their seed—both agricultural and genetic—from their homeland in the Near East as far west as Marseilles, but no farther.

To come to that conclusion, King disentangled seafarers’ complex migrations across the Mediterranean from 10,000 to 2000 years ago. He focused on the most common



genetic marker found in the Y chromosome of men living today in Anatolia and the Near East, called haplogroup J. The subgroup J2a is associated with people who traditionally lived in areas of higher rainfall and adopted agriculture rather than a semipastoral life.

Although the marker J2a appears across the Mediterranean, it is not necessarily a sign of a mass Neolithic migration. Much

later peoples, such as the Greeks and Phoenicians around 700 to 600 B.C.E., also spread from the Near East to the west—and their genes also include J2a. That makes it tricky to determine when the marker first appeared in the central and western Mediterranean. King, however, pinpointed markers specific to the ancient Greeks that were not shared with Neolithic people. Then he examined the

CREDITS (TOP TO BOTTOM): IMAGE COURTESY OF ISRAEL FINKELSTEIN AND STEVE WEINER (2); ADAPTED

tial burns. “It is a real surprise for all field archaeologists, who would reasonably have assumed that a fire occurred at the location,” Weiner said. Such “destruction events are generally regarded as ‘Pompeian’ ” and instantaneous, he said, but the new data challenges that view.

Other researchers have evidence from sediment cores that this arid region of the Levant became wetter in the 10th century B.C.E., providing expanded grazing during the period when Israelite tribes wandered north into the area. And residue analysis is opening a new window on possible religious practices of 3 millennia ago. Chalices found at Philistine sites such as Tell es-Safi include remains of a hallucinogenic substance that could be local artemisia or even nutmeg obtained from India. The residue is found in chalices across the region, despite the fact that they were locally made and often have differing decorations, a sign of a widespread, common ritual that until now was unknown.

Even critics of Weiner and Finkelstein were impressed by the battery of talks. The project is “a game changer” that is “stimulating a lot of us,” says archaeologist Thomas Levy of the University of California, San Diego, who thinks it will fundamentally alter biblical archaeology. Finkelstein says the team has 10 articles in the works, with more to come as DNA results arrive in the next year. Analysis of pig DNA is at the top of the list, he says, to show whether swine were an ancient local variety or were brought by sea peoples from the Mediterranean. —A.L.

population in the French city of Marseilles.

He found that 17% of the Marseilles population had Y chromosome input from the Greeks, and 18% carried Neolithic markers. This suggests a first wave of Neolithic settlers, followed millennia later by Greeks. But although the Greek genes spread far to the west, the Neolithic markers appear to stop in the Marseilles area, a sign that they ventured no farther. And because the markers are scarce inland, King says his results are yet more evidence that Neolithic farmers from the Near East spread to the region by sea.

Archaeologist Helen Farr of the University of Southampton in the United Kingdom says King’s study “adds compelling new DNA data to the evidence we have of the distribution and development of the Neolithic.” She adds that Neolithic seafaring is now well accepted and says what’s needed now is to carefully examine the material record at specific sites. —A.L.



Beachhead. This Egyptian fort, with reconstruction (below), was a key outpost.

Keeping Watch as the Old Kingdom Crumbled

With walls 7 meters thick and 4 meters high, the round stone fort was a potent symbol of ancient Egypt’s power, more than 250 kilometers from its Nile Valley heartland. And, so far, the building is unique. “We have no other example like it,” said excavator Gregory Mumford of the University of Alabama, Birmingham, after his presentation. But the structure at Ras Budran on the southern Sinai Peninsula also hints at the precariousness of Egypt’s Old Kingdom, suggesting an increasingly desperate trading and military strategy in the waning days of the 22nd century B.C.E.

Egypt’s Old Kingdom is one of the best known ancient cultures, thanks to hieroglyphic texts and the material culture buried in famous pyramids such as those at Saqqara and Giza. But the Old Kingdom was long thought to have been a relatively isolated society before its collapse in the 22nd century B.C.E., and little is known about Egyptian outposts from this time. “Not many fortified structures survived from the Old Kingdom, and Ras Budran offers a wonderful opportunity to learn more about the several types of fortifications” of the period, said Carola Vogel, an expert in Egyptian defenses at the Johannes Gutenberg University of Mainz in Germany.

Today, the Ras Budran fort is more than two football fields from the Red Sea. But back then sea levels were higher, and the fort likely stood at the water’s edge and included a lengthy wharf for access by seagoing ships.

Dated by pottery and radiocarbon to the end of the Old Kingdom, the structure paints a new picture of the society’s outer rim. Mumford’s team during the past three dig seasons has found copper ore, processed turquoise, and



copper chisels in the fort’s interior. The inhabitants made Egyptian-style pots from local clay and preferred a diet of bread and gruel while largely avoiding the local fish and mollusks. All this suggests that native Egyptians involved with valuable trade goods staffed the garrison. Ancient turquoise and copper mines are known in the area, and Mumford speculates that the fort may have protected precious wares from roving nomads before the material was shipped to the Nile. “Sinai was of utmost importance” at this time, says Vogel, for its resources and because it provided access to the Levant for trade.

Pictorial evidence from this period shows nomad attacks on Egyptian expeditions. Miniature clay models depict other forts from the era, but they are tall and narrow towers rather than the broad, open, and circular structure at Ras Budran. Stone also typically was reserved for monumental buildings like pyramids. And oddly, the limestone structure eventually was not just abandoned but partially and carefully disassembled by about 2200 B.C.E.

Shortly thereafter, a storm surge or seismic event destroyed much of what remained. A change of climate—increasing aridity combined with rising seas—plus increasing depredations by nomads likely prompted the withdrawal, said Mumford. Today, local Bedouin report a similar mound to the south, possibly the reconstructed fort on higher ground, he added. He intends to search for that site during next summer’s expedition.

—ANDREW LAWLER

FISHERIES

Chesapeake Crabs: Engineering a Rebound

A new strategy to selectively spare pregnant females has brought the Chesapeake Bay crab population back from a precipitous collapse in just 3 years

The first hint of dawn has barely leached over the Chesapeake Bay's horizon when Buddy Evans gaffs his first crab pot floater. Using a fast-spinning hydraulic winch, he hauls up the pot into his boat. His mate Jonathan Tyler shakes out some 30 startled blue crabs, mostly females with bright red claws. Tyler tosses the old bait to a posse of hysterical herring gulls, rebaits the pot—actually a rectangular yellow wire cage—and tosses it back into the water.

Below us, tens of millions of pregnant female blue crabs are walking up to 240 kilometers on invisible highways down to the mouth of the bay. Each will spend the winter there deep in the mud and then, between May and August, release between 750,000 and 5 million larvae. Then the females will move north into the bay to mate and return.

Eleven hours and 675 pots later, we roar home to Smith Island, the heart of the bay's 3-century-old community of watermen. The 36-foot boat's ample afterdeck is piled high with 88 bushel baskets filled with 13,000 crabs. It's 10 November, the last day of the fall female harvest season, and as night falls, we arrive in Crisfield, the hub of the crab business on the Eastern Shore peninsula, where the round wooden boxes are loaded onto a truck headed for New York.

"This is the best I've done in one day in years," says Evans. "I just wish we could do it for another 3 weeks, like we used to."

Across the bay at the University of Maryland Center for Environmental Science on Solomons Island, marine biologist Thomas Miller says that it's precisely by stopping harvesting early, when the fall female migration is at its peak, that one of the world's largest crab fisheries was brought from the brink of collapse to a healthy population in just 3 years.

For the second half of the 20th century, the Chesapeake's adult crab population oscillated around 400 million, with an average annual harvest of 250 million crabs. But in 1997, it declined to about 130 million, and up to 80% of these were caught each year. "You can't harvest 80% of anything and expect it to last," says Miller. "Getting people to fish less anywhere is very hard. Usually you keep on rearranging the deck chairs on the *Titanic* and when it starts to sink, you impose a fish-

ing moratorium, which is very disruptive to the communities."

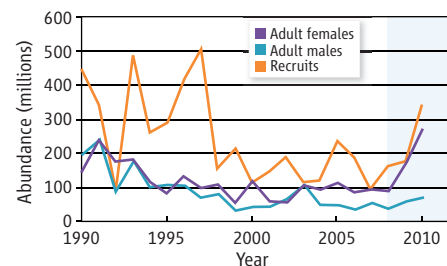
So how did Virginia and Maryland save the crab population from crashing? No thanks to Charles I. In 1632, he divided the bay, giving the northern half to Maryland and the rest to Virginia. "That's made it very hard to manage the Chesapeake as a single ecosystem," says Miller. "If Charles I only knew," says the British-born Miller's screensaver, over a portrait of the British monarch.

The solution emerged when Richard Robins, the owner of a seafood packing business with an MBA, was appointed to the Virginia Marine Resources Commission in 2004. He realized that the piecemeal efforts in both states to curtail the catch weren't working and called for a commission to determine why.

Miller was the senior scientist on the panel. He knew that 6 years of restrictions had only reduced the yearly catch from 80% to 63% of the adult population, not enough for it to rebound. The consensus on the panel was that they needed to focus the reductions on the female portions of the stock. "It was the kindergarten solution," Miller explains, so easy a 5-year-old could have figured it out: If there aren't enough babies, stop killing the mommies. "We needed to save at least half the number of pregnant females that were being harvested, about 30 million crabs," says Miller. "Overall, we wanted the harvest to be no more than 46% of the stock."

Miller had published an article in 2003 in the *Bulletin of Marine Science* demonstrating the benefits of sparing females. But because nearly 70% of them are caught in Virginia, near the mouth of the bay, it had never been tried in the Chesapeake because it was perceived as putting an unfair burden on Virginia watermen. "Without Robins taking the lead in Virginia, this approach would never have been acceptable," Miller says.

A broad array of measures was put in place in 2008; the shortening of the fall harvest of migrating females in both states has been the most effective. A winter dredge of buried hibernating pregnant females in Virginia—which killed two females for every one caught—was also closed. Those fishers were hired with federal funds to recover lost traps,



Big haul. Since the crab population in the Chesapeake Bay has rebounded, business is again booming for Buddy Evans (left) and Jonathan Tyler.

which continue to kill scores of crabs. Finally, a spawning-season sanctuary was extended, says Robins. "There are a lot of things that you can do to reduce mortality in a fishery," says Robins. "But this was the first time we used them all at once."

The watermen were not happy, but the spring 2009 survey of 1500 spots up and down the bay found that the female population had climbed by 70%, while the male population barely changed. This spring, the survey showed that the number of females was up 200% over the 2008 figure. Overall, the number of crabs has soared from 131 million in 2008 to 315 million this year.

"Given this year's catch, I expect we'll be very close to 400 million in the next spring survey," says Miller. "With two or three more years of increased reproduction, we shouldn't need to end the fall season so early, so the fishermen will catch more crabs in every pot and they can do it for longer."

"This brings the management of Chesapeake fishery closer to other crab fisheries, like the Alaska king crab and the west coast Dungeness crab, which ban the fishing of females," says David Armstrong of the University of Washington, Seattle. Unlike in the Chesapeake, females in those fisheries are significantly smaller than males, so a simple size restriction does the trick. "Still, a recovered, healthy fishery in the Chesapeake can certainly include some female harvest," Armstrong adds.

—CHRISTOPHER PALA

Christopher Pala is a writer in Washington, D.C.

CREDITS (TOP TO BOTTOM): C. PALA/SCIENCE; DATA COURTESY OF THE NOAA CHESAPEAKE BAY STOCK ASSESSMENT COMMITTEE

Downloaded from www.sciencemag.org on December 9, 2010

SCIENTIFIC COMMUNITY

Will Homebody Researchers Turn Japan Into a Scientific Backwater?

Few young Japanese scientists these days opt for long-term overseas experience; research leaders hope to find ways to broaden their horizons

CHIBA, JAPAN—When Rei Sakuma was completing his Ph.D. at the University of Tokyo in the mid-2000s, the theoretical physicist considered applying for overseas fellowships. His adviser talked him out of it. In Japan's shrinking academic job market, most positions are filled by word of mouth. Senior professors "do not want to hire strangers. If you're working in a foreign country, people in Japan forget about you," Sakuma says. Heeding his adviser, Sakuma took a postdoc in Japan and in 2008 landed a faculty position at Chiba University.

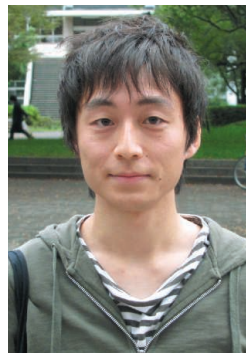
Staying home might have been a smart career move for Sakuma. But he became part of a trend: Fewer young Japanese scientists are going abroad for advanced training. "A lack of mobility leads to isolation at a time when our challenges [are] more and more global," says biochemist Ernst-Ludwig Winnacker, secretary general of the Human Frontier Science Program in Strasbourg, France. "We are very worried that without young researchers gaining overseas experience, Japanese science will fall behind," adds Kojiro Kakimoto of the Japan Society for the Promotion of Science (JSPS) in Tokyo. JSPS hopes to turn the tide with new programs that dispatch researchers abroad. But what's most needed, observers say, is a change in hiring procedures at home.

Recent surveys reveal a deepening reluctance of Japanese scientists to venture overseas. Last October, the education ministry reported that the number of researchers of all ages and in all fields who go abroad for longer than a month dropped from a peak of 7674 in 2000 to 3739 in 2009. More tellingly, in 2009 just 373 Japanese researchers were abroad for a year or more; of those, 47% were under 45 years old.

Other surveys affirm a stay-at-home tendency. According to the Institute of International Education in New York City, 24,842 Japanese students went to the United States for graduate and undergraduate education in the school year that ended in 2010, down from a peak of 47,073 in 1998. More Japanese might be going to other countries. But in 2009, the National Institute of Science and Technology Policy in Tokyo reported that of 60,535 Japanese who earned doctorates in Japan from 2002 to 2006, a mere 2%

are known to have gone abroad for postdocs or other opportunities.

Such immobility marks a sharp reversal from what was once the norm. Not long ago, "it was common understanding that if you didn't get a Ph.D. from an American university you couldn't get a job at University of Tokyo," says Takashi Shiraishi, a political scientist who studied and taught at Cornell University before returning to Japan. Budding researchers once had to go abroad to find suitable graduate programs, mentors,



Staying put. Like hordes of colleagues, Rei Sakuma opted for a postdoc fellowship in Japan rather than one overseas.

and state-of-the-art equipment—all of which now abound in Japan. And institutions once commonly sent newly hired science graduates abroad for further training; that rarely happens these days.

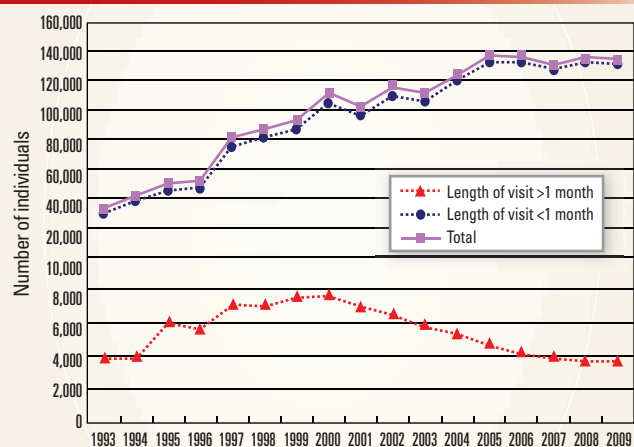
Other factors dampen academic wanderlust. Tight budgets and heavier teaching and administrative loads make it harder for faculty and staff members to go abroad for extended periods, says Sotaro Shibayama, a science policy researcher at the Georgia Institute of Technology in Atlanta. Meanwhile, a rapid expansion of graduate programs and postdoc fellowships since the mid-1990s is generating bumper crops of Japanese Ph.D. scientists who compete for a diminishing number of permanent positions.

The biggest restraint on going abroad may be cultural: Those who stay close to decision-makers seem to advance. Ken-ichi Arai, a

molecular biologist and University of Tokyo professor emeritus who worked and taught in the United States for 12 years, says that under the "hierarchical" Japanese system, senior professors steer positions to researchers they trained as Ph.D.s and postdocs. Scientists who have gone overseas "have a great deal of difficulty" fitting back into the system "unless they have a mentor in Japan," Arai says.

At the same time, scientists of all generations agree on the benefits of overseas experience. "Since Japan is isolated from other regions of the world culturally, geographically, and linguistically, I think it important for young Japanese people to spend some time abroad," says Hiroo Imura, an endocrinologist who spent 2 years as a postdoc at the University of California, San Francisco, in the early 1960s and went on to become president of Kyoto University, his alma mater, in the 1990s. Thanks to JSPS grants, Sakuma has attended conferences and made lab vis-

JAPANESE SCIENTISTS MAKING OVERSEAS VISITS



its abroad, including 2 months last summer at the Jülich Research Center in Germany. Longer visits would foster tighter collaborations and allow for stays at other institutions, Sakuma says, but "would be very difficult" because of his teaching duties.

Hoping to make headway against the prevailing winds, JSPS in October selected 96 doctoral students and young researchers for 1- to 3-year stints in overseas labs under a new grant scheme. And with JSPS and other organizations, the Human Frontier Science Program is planning a career day in April 2011 for junior Japanese scientists "to make them aware of the advantages of working and living overseas," Winnacker says. A lasting solution would be more-flexible career paths: "not just so young people can go abroad," says Arai, "but so they can come back."

—DENNIS NORMILE



LETTERS

edited by Jennifer Sills

Travel Trade-Offs
for Scientists

I RECENTLY RETURNED FROM THE ANNUAL meeting of the Ecological Society of America (ESA), my principal scientific society. This year's conference theme was "Global Warming: The legacy of our past, the challenge for our future." Roughly 4000 scientists attended from countries around the world to discuss the effects of greenhouse gas emissions on ecosystems and species. In so doing, we likely emitted more than 1000 tons of CO₂ (1). We are not alone. Other scientific societies, such as the American Geophysical Union and AAAS, also focus on climate change while holding large annual conferences. ESA purchased \$5 of carbon offsets for every attendee and implemented other green measures, but these measures do not go far enough.

Almost certainly, as individuals, scientists are responsible for an order of magnitude more greenhouse gas emissions than the average global citizen, in large part due to travel associated with our scientific interactions, presentations, and service to the scientific community (2). We warn about the serious consequences of "business as usual," yet we ourselves have failed to make necessary changes.

Science funding agencies and scientific societies should be the leaders in reducing greenhouse gas emissions from travel. There is undeniably great value in personal interactions at scientific meetings; scientists should explore ways to facilitate those productive collaborations without requiring travel. Science funding agencies can develop technological solutions to substitute for face-to-face panels and committee meetings. Social scientists can focus on maximizing our effective-

ness in scientific interactions over video conferencing. Funding agencies can encourage proposals that incorporate videoconferencing equipment, rather than large travel budgets. Scientific societies can develop strategies for less frequent national meetings, perhaps alternating with regional meetings, and advancing Web conferencing models.

Scientists must lead the charge against "business as usual" by demonstrating a different way of doing business. The technology is available, and it is time for us to find ways to promote scientific progress without contributing so substantially to the climate change problem we study. **INGRID C. BURKE**

Departments of Botany and Renewable Resources, Environment and Natural Resources Program, University of Wyoming, Laramie, WY 82072, USA. E-mail: iburke@uwyo.edu

References and Notes

1. Based on the ESA conference attendance list, I estimate that attendees traveled a total of about 8 million miles. I used the carbonfund.org carbon calculator to convert this estimated mileage to CO₂ emissions.
2. American Association for the Advancement of Science, "AAAS Board Statement on Climate Change" (2006); www.aaas.org/news/press_room/climate_change/mtg_200702/aaas_climate_statement.pdf.

Cataloguing Soil
Carbon Stocks

IN THEIR REPORT ("TERRESTRIAL GROSS Carbon dioxide uptake: Global distribution and covariation with climate," 13 August, p. 834), C. Beer *et al.* joined others (1, 2) in estimating global carbon fluxes and their relationship to climate. However, the global role of soil carbon stocks (i.e., the amount of organic carbon stored in the soils) and their sensitivity to climatic conditions have been largely ignored. This is a critical issue because there is more than twice as much carbon in the soil as in the atmosphere, and this carbon can be rapidly lost to the atmosphere when changes occur in climatic conditions and soil moisture. Moreover, most of the soil carbon is stored in the northern permafrost region (3, 4), one of the most vulnerable to climate change. The amount of carbon entering or leaving the system (net ecosystem exchange) may be quite different for ecosystems with large soil carbon stocks as compared to ecosystems with large aboveground biomass. Even though the global effect of temperature on ecosystem carbon fluxes has been reported [Beer *et al.* and (2, 5)], the influence of temperature on soil carbon stocks is less clear (6, 7).

To understand the effects of climate change on soil carbon stocks at a global scale, it will be necessary to develop a global database of soil carbon stocks. Various regional and national databases exist, but their utility and comparability are limited by differences in methodology (such as bulk density analysis, stone content analysis, and chemical analysis) and approach (such as sampling scheme and depth). Given the potential importance



Readers' Poll

Travel Trade-Offs for Scientists

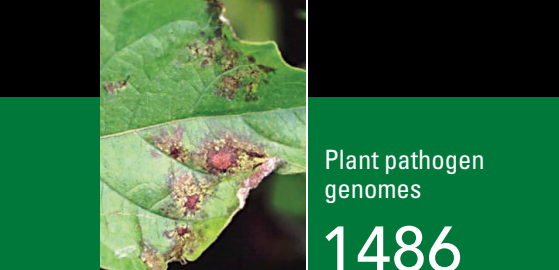
In her Letter, I. C. Burke urges scientists to examine the environmental consequences of their own frequent travel. She suggests that enhanced video-teleconferencing technology could effectively simulate face-to-face interactions, making alternatives to traditional conferences more feasible and in turn reducing greenhouse gas emissions by eliminating some travel to meeting sites.

Would you participate in an annual meeting remotely (via video teleconferencing or other technology)?

- ☐ **Yes: Participating remotely would be about as valuable as attending in person.**
- ☐ **Yes: It would lose some value, but the trade-off would be acceptable given the environmental benefits.**
- ☐ **No: It would lose some value, and the trade-off would be unacceptable despite the environmental benefits.**
- ☐ **No: Participating remotely would be about as valuable as not attending at all.**

Vote online at www.sciencemag.org/extra/polls/20101210-1.xhtml

Polling results reflect the votes of those who chose to participate; they do not represent a random sample of the population.



Plant pathogen
genomes

1486



GWAS and AIDS

1488



Overlooked. Few carbon flux analyses account for soil carbon stored in permafrost.

of changes in terrestrial soil carbon stocks to atmospheric carbon chemistry and global warming, it seems appropriate and necessary to develop reliable global evaluation of current carbon storage and estimates of likely changes in the future. This will require an international collaboration similar to the coordinated effort that produced the Fluxnet, an international flux measurement and modeling network (8).

DAMIANO GIANELLE,¹ WALTER OECHEL,²
FRANCO MIGLIETTA,³ MIRCO RODEGHIERO,¹
MATTEO SOTTOCORNOLA¹

¹Research and Innovation Centre, Environmental Area, Fondazione E. Mach, San Michele all'Adige Trento, 38010, Italy.
²San Diego State University, San Diego, CA 92182, USA.
³Istituto di Biometeorologia, CNR-IBIMET, Firenze, 50145, Italy.

*To whom correspondence should be addressed. E-mail: damiano.gianelle@iasma.it

References

1. M. Jung *et al.*, *Nature* **467**, 951 (2010).
2. C. Yi *et al.*, *Environ. Res. Lett.* **5**, 034007 (2010).
3. E. A. G. Schuur *et al.*, *Biosciences* **58**, 701 (2008).
4. C. Tarnocai *et al.*, *Global Biogeochem. Cyc.* **23**, GB2023 (2009).
5. B. Bond-Lamberty, A. Thomson, *Nature* **464**, 579 (2010).
6. S. E. Trumbore, C. I. Czimczik, *Science* **321**, 1455 (2008).
7. P. Smith, F. Changming, *Nature* **464**, 499 (2010).
8. D. Baldocchi, *Aust. J. Bot.* **56**, 1 (2008).

Credit to Plowright for Rinderpest Eradication

I WAS DISAPPOINTED THAT THE NAME OF Walter Plowright was not mentioned in the News of the Week story "Rinderpest, deadly for cattle, joins smallpox as a vanquished disease" (D. Normile, 22 October, p. 435). In the late 1950s, Plowright used the newly emerging techniques of tissue culture to serially passage the pathogen such that it lost its ability to cause disease but retained its antigenicity (1). The resulting live vaccine produced a lifelong immunity to the disease. He therefore became the equivalent of Jenner in the eradication of smallpox. In recognition of his achievements, Plowright was elected to the Royal Society in 1981 and received the World Food Prize, which honors advances in human development, in 1999. Unfortunately, he died in February of 2010 before the FAO announced the eradication of the disease, which has a huge impact on animal production, especially in Africa.

DAVID ROBERTSHAW

Department of Biomedical Sciences, Cornell University, Ithaca, NY 14850-6401, USA. E-mail: dr11@cornell.edu

Reference

1. W. Plowright, *J. Hyg. Camb.* **92**, 285 (1984).

Optimizing Scientific Reasoning

B. BAHRAMI *ET AL.* ("OPTIMALLY INTERACTING minds," Reports, 27 August, p. 1081) found that social interactions can enhance the quality of decision-making. The Perspective by M. O. Ernst ("Decisions made better," 27 August, p. 1022) suggested that Bahrami *et al.*'s results could be applied to real-world issues such as soccer refereeing. I propose another application: the nature of scientific reasoning.

Science is the collective selection of theories given available evidence and Bayesian probabilistic deduction (1). But scientists reason in ways that go beyond the requirements of Bayes. For example, they collectively work to ensure that scientific ideas are stated clearly, exposed to open criticism, and

judged purely on their merits (blind review). Bahrami *et al.*'s research sheds light on why such norms are central to science.

Bahrami *et al.* found that inferences made collectively by a group were superior to inferences made with only individual judgment. However, some social interactions were more effective than others in arriving at a correct decision. Science is the accumulated product of many centuries of experience in how best to organize research communication to optimize its capacity for collective Bayesian inference. Bahrami *et al.*'s experimental paradigm, although not directly a science reasoning task, provides a means by which these norms central to scientific reasoning can be researched and perhaps even improved.

JOHN R. SKOYLES

Centre for Mathematics and Physics in the Life Sciences, University College London, London, UK. E-mail: j.skoyles@ucl.ac.uk

Reference

1. C. Howson, P. Urbach, *Scientific Reasoning: The Bayesian Approach* (Open Court, La Salle, IL, 1989).

CORRECTIONS AND CLARIFICATIONS

News Focus: "Scientific gold mine or dicey money pit?" by A. Cho (12 November, p. 904). The story misstates the primary affiliation of Kevin Lesko, principal investigator for the proposed Deep Underground Science and Engineering Laboratory. His primary affiliation is the University of California, Berkeley, not Lawrence Berkeley National Laboratory.

Reports: "SNP genotyping defines complex gene-flow boundaries among African malaria vector mosquitoes" by D. E. Neafsey *et al.* (22 October, p. 514). The authors mistakenly failed to include an acknowledgment in support of author G. K. Christophides: "the European Commission FP7 Collaborative project grant HEALTH-F3-2008-223736 to G.K.C."

News of the Week: "Custom-built supercomputer brings protein folding into view" by R. F. Service (15 October, p. 308). The last sentence of the first paragraph was incorrect. The sentence should have read: "They also tracked the gyrations of a similarly sized folded protein for more than a millisecond."

News Focus: "Grave disputes" by A. Lawler (8 October, p. 166). Keith Kintigh of Arizona State University in Tempe was mistakenly identified as a physical anthropologist. He is an archaeologist and former president of the Society for American Archaeology.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

Science Books for Fun and Learning—Some Good 2010 Choices

If you are trying to encourage an interest in science among children or young adults, the finalists for the 2011 Science Books and Films Prizes for Excellence in Science Books should provide some possibilities for your holiday gift list. The prizes honor works that promote an understanding and appreciation of science in younger readers. Sponsored by AAAS and Subaru, they are awarded in four categories: children's science picture book (for readers in grades K to 4), middle grades science book (grades 5 to 8), young adult science book (high school), and hands-on science/activity book (any age). As in recent years, most finalists for the young adult award were not specifically intended for that age group but were written for the



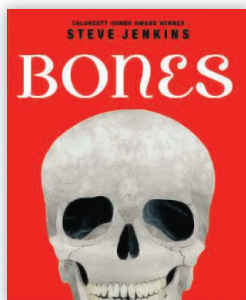
general public. The titles considered for the 2011 prizes were published between September 2009 and August 2010.

Here, we offer short descriptions of the 16 finalists chosen by panels of librarians, educators, and scientists. Full reviews of each book have been published or will appear in *Science Books and Films*, and AAAS members can read these reviews on the Web. The winners will be announced in February at the AAAS Annual Meeting in Washington, DC.

The clear and accurate presentation of scientific concepts is one of the criteria used by the judges. But we hope young readers will find the books both informative and enjoyable.

—Heather Malcomson,¹ Barbara Jasny, Laura Zahn, and Sherman Suter

Children's Science Picture Book



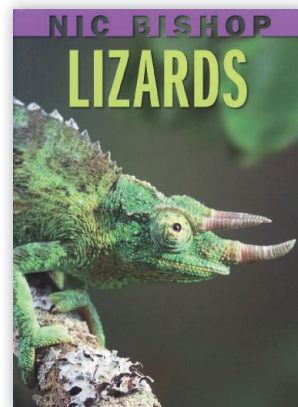
Bones. Steve Jenkins. Scholastic, New York, 2010. 48 pp. \$16.99. ISBN 9780545046510. Surprisingly, the precisely scaled, mottled white, gray, and dusty yellow illustrations of bones that fill this book's pages are not photographs or drawings but cut-paper collages. Jenkins uses them to show variations and similarities among elements of vertebrate skeletons such as hands, feet, limbs, ribs, and backbones. Several animals are presented as full skeletons, sometimes in active (or even humorous) poses: a rabbit

bounding away from a pouncing leopard, and a person pursued by a charging rhinoceros. A small python, with nearly 200 ribs, occupies a four-page foldout. Another foldout offers 11 skulls (ranging from human and wild pig to tree shrew and chameleon), also at their actual sizes. A pair of facing pages titled "Some assembly required" shows all 206 human bones organized into groups; opening the pages reveals the complete skeleton. The limited text touches on adaptations, symmetry, and joints, and a brief section at the back of the book introduces a hodgepodge of scientific facts about bones.

Lizards. Nic Bishop. Scholastic, New York, 2010. 48 pp. \$17.99. ISBN 9780545206341.

Most of the world's 5000 species of lizards inhabit warm climates such as tropical rain forests and hot deserts. They range in size from the tiny dwarf gecko of the Caribbean to the giant Komodo dragon of Indonesia. Colorful closeup, full- and double-page photographs (with small species portrayed

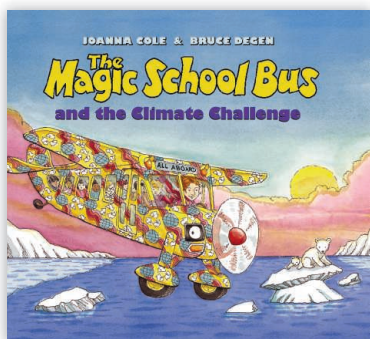
at two to four times actual size to capture their character) introduce examples such as the legless glass lizard, skinks, monitors, and the sandfish that swims through dunes. Other images show lizards in action: a veiled chameleon extending its tongue nearly 30 cm to capture an insect prey, a gliding gecko sailing through trees on its webbed feet and skin flaps, and a basilisk sprinting across water. Bishop, who holds a doctorate in biological sciences, writes clearly on a range of topics: life history, behavior, physiology, diets, hunting tactics, defense against predators, camouflage, courtship, and reproduction. In an informative epilogue, he describes his efforts to find a thorny devil in the outback of Western Australia and his experiences photographing animals in the wild and in captivity.



The Magic School Bus and the Climate Challenge. Joanna Cole, illustrated by Bruce Degen. Scholastic, New York, 2010. 48 pp. \$16.99. ISBN 9780590108263.

For more than 20 years, Cole and Degen have been writing and illustrating their series about the amazing teacher, Ms. Frizzle, who takes her class on fantastical adventures that teach readers about scientific principles while being vastly entertaining. In this latest installment, the school bus changes into a plane that whisks the class off to the Arctic and other parts of the world to see the changes that have been occurring as a result of global warming. Student "reports" at the edges of the pages provide facts about the science and the choices that are being faced. (For example, one describes the pluses

¹Science Books and Films. E-mail: hmalcoms@aaas.org

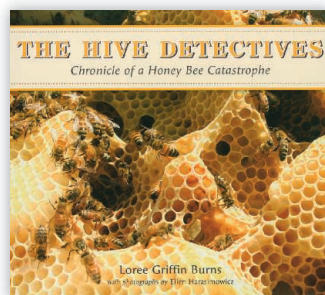


on a play about global warming for their community. As with the earlier adventures in the series, the whimsical illustrations are a big part of the book's success.

and minuses of biofuels.) The students learn about the greenhouse effect first-hand when they slide down to Earth on sunbeams and are wafted back up as heat—only to bounce off the greenhouse gases and descend to Earth again, flying through carbon dioxide emissions. At the end of the book, the students discover ways that they can save energy and put

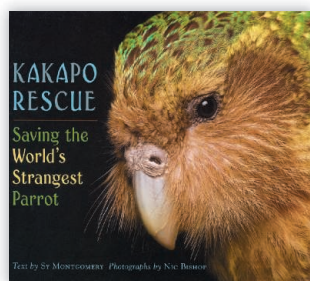
Middle Grades Science Book

The Hive Detectives: Chronicle of a Honey Bee Catastrophe. Loree Griffin Burns; photographs by Ellen Harasimowicz. Houghton Mifflin, Boston, 2010. 80 pp. \$18. ISBN 9780547152318. Scientists in the Field. Burns spotlights an unsolved mystery: the cause of the catastrophic loss of honeybees to “colony collapse disorder” (CCD). She first introduces readers to beekeeping by following a hobbyist as she inspects her beehives. The author then tells how commercial beekeeper Dave Hackenberg, checking 200 of his hives in Florida, found that 2 million of his pollinators had vanished. In subsequent sections, she describes the work of four scientists whose specialties (autopsies, bee pests such as mites, bacterial and viral pathogens, and pesticide monitoring) opened separate lines of research to consider different possible explanations for the threat to bees and, by extension, agriculture. Burns summarizes the researchers’ procedures, equipment, and findings before reporting the theory that the outbreak of CCD may stem from a novel combination of factors. Special feature pages and appendices present aspects of honeybee anatomy, development, and social behavior along with how beekeepers harvest honey and the crucial roles bees play in pollinating plants and crops.

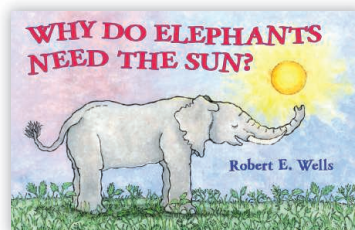


The color photographs included on nearly every page effectively complement the informative text.

Kakapo Rescue: Saving the World's Strangest Parrot. Sy Montgomery; photographs by Nic Bishop. Houghton Mifflin, Boston, 2010. 80 pp. \$18. ISBN 9780618494170. Scientists in the Field. On a small island off southern New Zealand, the author and the photographer spent 10 days with conservation biologists



and volunteers who are attempting to stave off the extinction of the kakapo (*Strigops habroptilus*). The heaviest of parrots (males weigh up to 3 kg), kakapo are terrestrial, flightless, and nocturnal. Although the species was formerly common across New Zealand, introduced weasels, stoats, and cats eliminated natural populations. A few score survivors were translocated to predator-free islands, but by 1995 only 51 birds remained. Montgomery and Bishop accompany members of the recovery team as they monitor



Why Do Elephants Need the Sun? Robert E. Wells. Albert Whitman, Park Ridge, IL, 2010. 32 pp. \$16.99. ISBN 9780807590812.

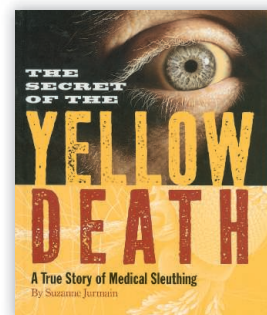
This playful but substantive book uses a fun story to show the inter-

connectedness of the natural world and, in particular, the many roles played by the Sun. Wells introduces young readers to our closest star and its important functions and uses (including gravity, nuclear fusion, photosynthesis, telling time, and generating electricity). His accessible presentation features an elephant who needs the Sun for warmth, food, and even water (evaporated from the sea and blown inland to fall as rain or snow). Full of the author's kid-friendly drawings and diagrams, the book should help children begin to understand several, sometimes abstract, scientific concepts.

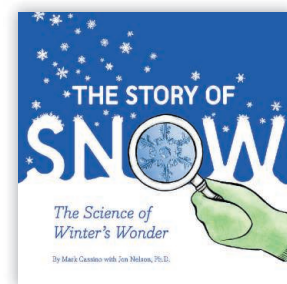
nesting burrows using hidden cameras, employ handheld receivers and automatic recorders (“snarks”) to radio-track bird movements, weigh chicks, provide carefully selected and handled foods at outdoor stations, and even incubate eggs gathered from some nests. Their visit included both lows (deaths of chicks and of a male at least 70 years old) and highs (hatchings in nests and in the lab; an unexpected daytime encounter). Text and photographs give readers an eyewitness account of the hands-on measures being taken on Codfish Island to save the green, honey-scented birds. By the end of the season, the recovery team's substantial investment of resources and time had been rewarded.

The Secret of the Yellow Death: A True Story of Medical Sleuthing. Suzanne Jurmain. Houghton Mifflin, Boston, 2009. 112 pp. \$19. ISBN 9780618965816.

In a book that conjures memories of *The Microbe Hunters*, Jurmain recounts work of scientists and volunteers from Cuba and the United States to find the cause of yellow fever. She graphically describes symptoms (and the death of a researcher) as well as the frustrations of the scientists, who had to painstakingly work their way through prevailing incorrect hypotheses (that the disease was caused by poisonous gas or transmitted via infected bed-clothes) to show that the bite of an infected mosquito was the culprit. The excellent writing and photos from the early 1900s (when the investigation was carried out) bring the researchers to life, and the clear presentation of the scientific method draws the reader into the story. An epilogue reminds us that yellow fever remains a problem in parts of Africa and South America.



The Story of Snow: The Science of Winter's Wonder. Mark Cassino, with John Nelson. Chronicle, San Francisco, 2009. 36 pp. \$16.99, £10.99. ISBN 9780811868662. It seems hard to go wrong with a book about snowflakes, and this combination of drawings, photos, and simple explanations proves no exception to the rule. Cassino clearly explains the formation, structure, and individuality of snowflakes. Microphotographs in grays and blues depict the range of crystal forms—six-armed stars, plates, and columns. The lovely images of delicate structures, twins, multicrystal flakes, and “bumps” of water droplets (rime) on a snow crystal will entice readers to try the authors’ method to “catch your own snowflakes.”



Young Adult Science Book

The Case for Pluto: How a Little Planet Made a Big Difference.

Alan Boyle. Wiley, New York, 2009. 272 pp. \$22.95, £15.99.

ISBN 9780470505441.

In 2006, the International Astronomical Union voted to downgrade Pluto to the status of a "dwarf planet." The decision was greeted with strong criticism from many astronomers, an outcry from interested members of the public, and even "hate mail" from some third-graders. Science writer Boyle presents

the research, personalities, and politics involved in our understanding of the icy world. He begins by recounting Percival Lowell's misguided search for Planet X and how that led to Clyde Tombaugh's 1930 discovery. In recent decades, improved observational technologies have helped reveal more about the nature of Pluto, the existence of its relatively large moon Charon, and the presence of two additional tiny moons. Astronomers peering at the distant fringes of the solar system also discovered the Kuiper Belt with many mini-planets (and at least one member, now known as Eris, that is larger than Pluto and has its own moon). The question of how to classify such objects fueled a contentious debate over what is a planet.

Boyle accurately summarizes the positions

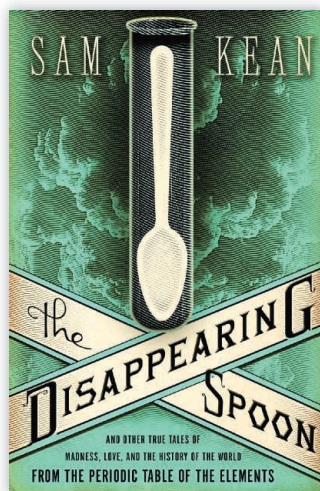
of "geophysicists" (who see the critical trait as possessing sufficient gravity to form a "nearly round" shape) and the dynamicists (who place more emphasis on the object's gravitational effects on other bodies). But his lively and informative account is clearly biased toward those who would restore Pluto to the position of the Sun's ninth planet.

The Disappearing Spoon: And Other True Tales of Madness, Love, and the History of the World from the Periodic Table of the Elements.

Sam Kean. Little, Brown, New York, 2010. 400 pp.

\$24.99, £20. ISBN 9780316051644.

One of the great scientific achievements, the periodic table of elements provides an organizing principle for much physical science. Kean's informative and engaging book amply demonstrates that it can also provide a starting point for stories involving history, politics, literature, and art as well as a wide range of human passion, adventure, folly, and treachery. All of his accounts have some connection, at times indirect, to the periodic table as a whole or to particular elements. Many concern well-known scientists, their accomplishments, and their lives or describe other interesting aspects of science that are familiar at least by name to the general public. Kean writes in a brisk, jaunty style and often uses analogies from daily life. When appropriate, he segues from anecdotes to the chemistry behind them. His lucid explanations of science should be accessible to high school students, although readers will find some prior acquaintance with the periodic table and atomic theory useful.



Every Bone Tells a Story:

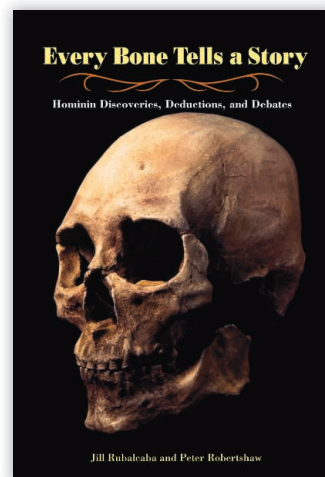
Hominin Discovery, Deductions, and Debates. Jill Rubalcaba and Peter Robertshaw. Charlesbridge, Watertown, MA, 2010.

185 pp. \$24.95, £11.95.

ISBN 9781580891646.

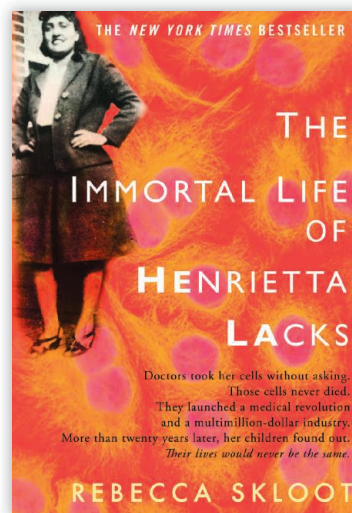
The authors present the fascinating stories of four of our ancient relatives: Turkana boy (from 1.6 million years ago in Kenya), Lapedo child (24,000 years ago, Portugal), Kennewick man (9000 years ago, Washington state), and Ötzi the iceman (5300 years ago, the Alps). They divide each narrative into sections that cover the field discoveries

of the hominin remains and their contexts, subsequent deductions from laboratory work, and the debates over interpretations of the findings. At the end of each story, the authors suggest further readings and relevant Web sites and provide source notes for their meticulous accounts. Rubalcaba and Robertshaw clearly present the thought processes that scientists use to reach their conclusions, introduce the scientists as people while illustrating why they are so excited about their research, and accurately describe the scientific data for the reader's evaluation. Exceptionally well written, the book provides an exciting read that makes the joy of being a scientist come alive.



The Immortal Life of Henrietta Lacks. Rebecca Skloot. Crown, New York, 2010. 284 pp. \$26. ISBN 9781400052172. Macmillan, London. £18.99. ISBN 9780230748699.

The ubiquitous cell line HeLa (whose immortality provides the book title) has helped power the explosive growth of cell biology over the past 50 years. But for all that is known about the cells themselves, most people know little about their original source. Skloot explores that origin and the subsequent confusion over these cervical cancer cells' history. She eloquently interweaves stories of the cells and researchers, the life of Henrietta Lacks (from whom the cells were cultured), and the response of Lacks's family to her investigation.



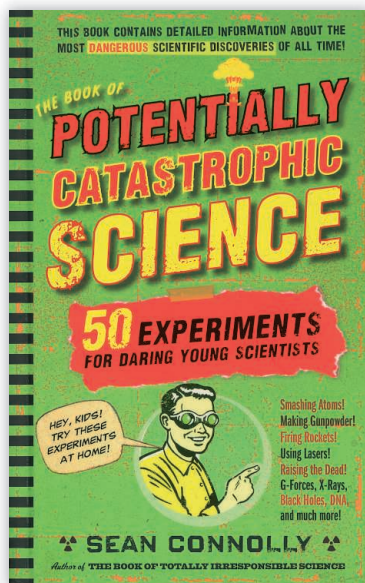
Carefully crafted, her rich and balanced narrative reminds us to not lose sight of the source of biological research materials. Interestingly, the author does so without passing judgment on the families' reactions (some of Lacks's relatives believe they should be paid for the gains made from the contribution she unknowingly made) or the researchers (some of whom could be said to have been dismissive of Lacks in their treatments of her and her cells). Skloot recognizes that research standards have changed over time and we have progressed in our treatment of patients. But the tale

that she so superbly tells provides anyone interested in biomedical science a timeless and necessary reminder of the humanity shared by the afflicted and the researchers who work to understand and overcome diseases.

Hands-On Science Book

The Book of Potentially Catastrophic Science: 50 Experiments for Daring Young Scientists. Sean Connolly. Workman, New York, 2010. 306 pp. \$13.95. ISBN 9780761156871.

This book stands out from the crowd of guides to science experiments that can be performed at home. Whereas many such works present a hodgepodge of standard experiments, Connolly builds this one around the theme of major scientific and technological breakthroughs that have occurred over the past 2-plus million years of human history—arranged from the first stone tools crafted by *Homo erectus* to the Large Hadron Collider now being used to accelerate particles to speeds approaching that of light. Each of the 34 chapters comprises descriptions of an advance and its context, the science behind it, and one or more experiments that demonstrate underlying principles. These are presented in a breezy and engaging style. Although calling the discoveries potentially catastrophic is sure to intrigue a certain kind of young experimenter, the author's explanations note both the benefits and the drawbacks of the advances. Parents will appreciate

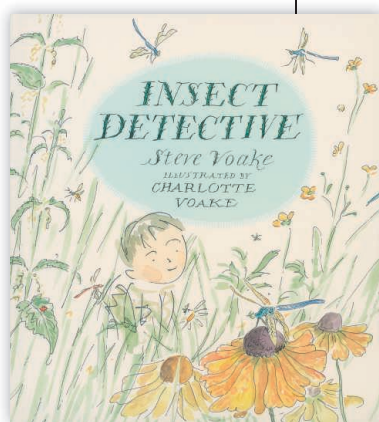


the “catastrophe meter” reading for each experiment, which indicates the hazards involved and the appropriate level of adult supervision. The experiments and their ties to the science are consistently creative: One can spool DNA onto a skewer after isolating it from a half-eaten banana. A handmade oven works because of some of the principles behind a laser beam. The speed of light can be estimated using a microwave oven, marshmallows, and a ruler. Even adults who have survived many science fairs will find themselves tempted to try some of Connolly's experiments.

Insect Detective. Steve Voake, illustrated by Charlotte Voake.

Candlewick, Somerville, MA, 2010. 32 pp. \$16.99. ISBN 9780763644475. Walker, London, 2009. £11.99. ISBN 9781406310511.

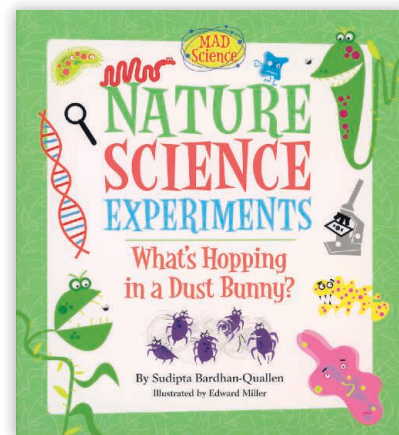
The author and illustrator (cousins) invite young readers to step outside to observe “thousands of insects...doing strange and wonderful things.” The conversational main text introduces a variety of creatures in their habitats, while occasional lines in small print provide interesting details and various facts. The Voakes feature insects one can find close to home: wasps, ants, solitary bees, moths, leaf-mining caterpillars, ground beetles, earwigs, and dragonflies. (Noninsects that one might also encounter can be identified because they don't have six legs.) Simple, informative ink-and-watercolor illustrations depict the animals engaged in typical behaviors. The book ends with short descriptions of seven activities for detectives who wish to learn more about their neighborhood insects.



Nature Science Experiments: What's Hopping in a Dust Bunny.

Sudipta Bardhan-Quallen, illustrated by Edward Miller. Sterling, New York, 2010. 64 pp. \$12.95, £9.99. ISBN 9781402724121. Mad Science.

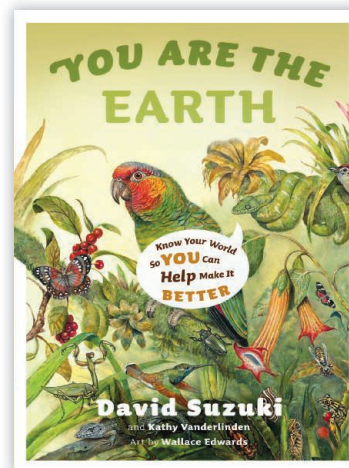
The baker's dozen intriguing activities in this book involve topics such as DNA from cheek cells, the hiding places of dust mites, and the trigger hairs of Venus flytraps. The author starts by presenting eight steps toward becoming a young scientist: asking questions, observing, being prepared, being informed, taking suggestions, getting assistance, being careful, and having fun. She introduces the simple experiments not with hypotheses but with general questions concerning their topics. The clear explanations will guide children through each step of the activities, and “What's going on?” sections discuss the expected outcomes. The straightforward experiments provide a wonderful starting place for hands-on inquiry learning in the classroom or at home.



You Are the Earth: Know Your World So You Can Help Make It Better. David Suzuki and Kathy Vanderlinden, illustrated by Wallace Edwards.

Greystone, Vancouver, Canada, 2010. 144 pp. Paper, \$16.95, C\$22.95. ISBN 9781553654766.

The author, a well-known Canadian environmental activist, combines science, enjoyable activities, and stories to explain the connections linking everything on Earth. Incorporating content that varies from scientific to the personal to cultural—with folk tales juxtaposed next to scientific evidence—the book entices one to keep reading just to see what is on the next page. Sections cover topics one expects to find in an environmentally oriented book, such as air, water, soil, heat, and the effects of human actions. In addition, Suzuki includes in every section stories of how people from different cultures live and use resources, examples of interactions among organisms and the environment, and structured opportunities for personal reflection. Throughout the book, he raises thought-provoking questions and suggests activities that could be done individually or as classroom demonstrations.



10.1126/science.1199722

For Younger Readers

Two from China

Jianguo Liu¹ and Shuxin Li

This year, we feature a pair of science books for children and young adults from China. These were selected from recommendations provided by colleagues in the Ministry of Education, China Association for Science and Technology, CCTV (China Central TV), China Association of Popular Science Writers, and several major publishers. Both titles represent popular series that are intended to introduce young readers to nature, science, and technology, and both books are suitable for students from the upper levels of elementary school through high school.

The most popular of such series is *A Hundred Thousand Whys*, which has influenced several generations of readers since it was started in the early 1960s. The books use a question-and-answer format to cover subjects such as astronomy, botany, geography, medicine, social sciences, and zoology. The series includes many picture books, and the material has also been released in video and CD sets.

The president of the Chinese Academy of Sciences (Yongxiang Lu) and other leading researchers edit another prominent series, *Approaches to Science*. Drawing material from a program on the educational channel CCTV-10, it explains scientific issues behind hot topics in the news and important societal problems.

潜海世界 (Qianhai Shijie) [Undersea World]. Yi Chen. Popular Science Press, Beijing, 2009. 107 pp. Paper, ¥29.90. ISBN 9787110069929. [Knowledge Is Power]. As an enthusiastic diver, Chen brings readers along on her exciting underwater journeys through relatively unknown and remote places in China and many other parts of the globe (such as Africa and the Philippines).

Center for Systems Integration and Sustainability, Michigan State University, East Lansing, MI 48824, USA. ¹E-mail: liuji@msu.edu



Color photos illustrate a variety of marine fauna described in the text, including nudibranchs, crabs, sea snakes, corals, fish, turtles, and sharks. There are also interesting and historic human-made objects and treasures—for example, a section of the Great Wall that has been hidden by water since the construction of a large reservoir in Hebei province several

decades ago. Through the text, the author also shares anecdotes regarding the challenges, surprises, and processes of her discoveries.

蜜蜂的故事 (Mifengdegushi) [Stories of Bees]. Jingdong Wang. Hubei Children's Press, Wuhan, Hubei, China, 2009. 214 pp. Paper, ¥10.80. ISBN 9787535345097. [Famous Popular Science Writers for Juveniles and Children].

Throughout the four decades of his adult life as a biology teacher in rural China, Wang has raised bees at home and observed them daily. Reflecting his passion and devotion, the award-winning author details alluring aspects of bee biology, such as social structure, division of labor, "marriage," communication, memory, architectural prowess, and honey production. He discusses several of the services bees provide people, including how the insects help produce fruits, detect mines, forecast weather, and enhance human health. Wang also tells fascinating stories about complex interactions among bees, other animals, plants, and microorganisms. Black-and-white diagrams and full-page drawings enhance the text.

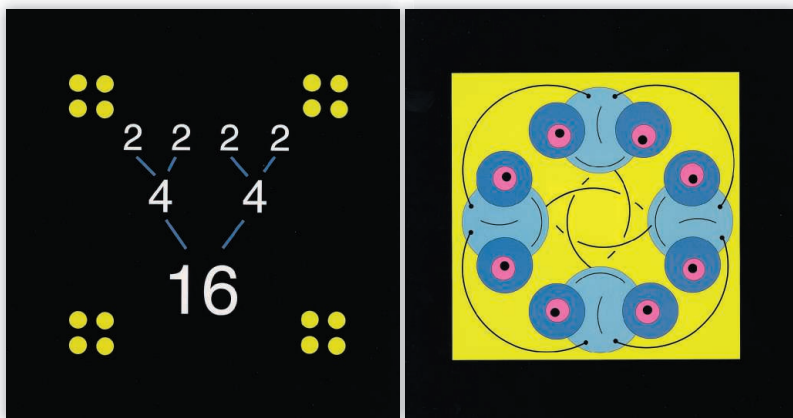


10.1126/science.1200647

BROWSINGS

You Can Count Monsters: The First 100 Numbers and Their Characters. Richard Evan Schwartz. A K Peters, Natick, MA, 2010. 241 pp. Paper, \$24.95. ISBN 9781568815787.

This parade of 100 monsters offers young children a playful visual exploration of multiplication and "the building blocks of numbers." Bright, colorful creatures represent prime numbers. Mathematician Schwartz illustrates composite numbers by combining the monsters that match their prime factors. For each number, he also provides a factor tree and an arrangement of colored dots that demonstrates its nature. Background information in the introduction and appendices will help older readers answer questions about factoring from the book's intended young audience.



10.1126/science.1199722

GLOBAL HEALTH

Stagnant Health Technologies in Africa

Ken Simiyu, Abdallah S. Daar, Peter A. Singer*

In Madina village, outside Accra, Ghana, children tease each other about whose urine has a redder color. Apart from being strikingly thin, they look healthy. Yet they could be affected by *Schistosoma haematobium* (1), a parasitic disease common in Africa, where local prevalence rates can exceed 50% (2). Early diagnosis ensures inexpensive and effective treatment and prevents stunted growth and developmental disabilities in children and bladder cancer or other organ damage in adults (3). But the standard method of detecting the disease, microscopic identification of eggs in urine or stool, requires patients to visit a hospital laboratory, something not practical for many people living in rural Ghana.

A solution to this problem could be just 20 km away, at the Noguchi Memorial Institute for Medical Research in Accra, where a low-cost point-of-care rapid monoclonal antibody-based dipstick technology has been developed to diagnose the disease. This visually read test can be used at the village level. Although this work has been published (4), the technology, which has the potential to save lives, has not been commercialized; instead, it lies stagnant.

Unfortunately, this situation is replicated in many of sub-Saharan Africa's health research institutions where many technologies are being researched but not commercialized, that is, converted to a product or service that generates profits or has an impact on the everyday lives of Africans (5).

To better understand why this is happening, we visited 23 academic and health research institutes in Ghana, Kenya, Nigeria, Rwanda, Tanzania, and Uganda and interviewed 39 scientists. We asked respondents to identify "technologies that have been developed or are being developed, but have failed to attain commercialization for one reason or another" [see supporting online material (SOM) for study details] (6), a phenomenon we refer to as "stagnant technolo-



An African health technology awaits commercialization. Moses Musaaizi of Makerere University and the medical-waste incinerator he invented.

gies." We discuss here this problem and some approaches to addressing it. Previous studies in Africa have examined health innovation at the country level (7), but to our knowledge, there are no systematic studies of stagnant health technologies. This article highlights some of these technologies and why they stagnate, as described by the African researchers themselves.

Stagnant Technologies

We identified 25 technologies, including traditional plant products, new drug molecules, diagnostics, vaccines, and medical devices (see SOM). Many of the technologies identified (16 out of 25) were products of traditional plant medicines. Examples include an antimalarial product, Nibima, from a traditional plant *Cryptolepis sanguinolenta* (8), which is being developed by scientists at the Centre for Scientific Research into Plant Medicines, in Mampong, Ghana; and Sunguprot from the plant *Tylosema fassoglensis*, whose developers in Kenya claim can be used to manage HIV symptoms. Lack of advanced scientific equipment to isolate compounds or funds to carry out clinical trials have affected further development and validation.

Researchers at Tanzania's National Institute of Medical Research are working on alternative methodology to extract and purify

Commercializing technologies may help alleviate some of sub-Saharan Africa's health and economic problems.

artemisinin from *Artemisia annua* and to prepare derivatives or combine it with other antimalarials to combat the emerging problem of artemisinin resistance. But they lack a larger machine to produce enough extract to carry out clinical trials and facilities for structural analysis of drug molecules.

Other scientists are working on developing point-of-care diagnostics. At the University of Ghana, researchers are developing a visually readable portable dipstick test that uses monoclonal antibodies to detect the malaria parasite in urine. Work at Makerere University in Uganda focuses on developing a rapid diagnostic test for extremely drug-resistant tuberculosis. But the focus has not been on commercialization. As one of the scientists we interviewed said, "I have developed this

rapid-detection test which has shown positive results in the lab, but my motive was never to develop a product."

Scientists are also developing medical devices and equipment. Notable ones identified in this study include a fuel-free medical-waste incinerator (see the photo), developed at Makerere University, which is cheap to purchase and operate, portable, and suitable for use in rural settings. This could provide a solution to management of hospital waste in rural areas, especially for programs such as mass polio immunization. At the International Centre for Insect Physiology and Ecology in Kenya, researchers have patented human odors that are effective in repelling mosquitoes. However, there is need for further research to determine formulations, though negotiations are ongoing with a multinational company.

Although these examples, which are at different stages of development, may not be representative of every institution and country in sub-Saharan Africa, they illustrate the scope and extent of technology stagnation.

Why Do These Technologies Stagnate?

Although bringing technologies from laboratory to village faces scientific, ethical, commercial, and political barriers (9), our focus here is on barriers to developing validated

commercial products. Among the key reasons why technologies stagnate is the cultural mindset of scientists and domestic and international policy-makers whose focus is not commercialization. As in the rest of the world, academic researchers are typically judged by publications rather than economic impact or lives saved. As one scientist put it, “My primary focus is to teach and publish. Someone else can take up products to develop them if they are interested, as no one will recognize my effort.”

Lack of institutional mechanisms to support further validation and subsequent commercialization exacerbates the problem. For example, there are few “innovation funds” to support proof of concept and prototype development, such as the millennium fund in Uganda (10) and the innovation fund in Kenya (11). Because of inherent uncertainties of life sciences, it may be that most funds end up going to fields like engineering. Venture capital (VC) is not widely available. Although there have been funding pledges—for example, in 2007, African Union heads of state committed to devoting 1% of their gross domestic product to science and technology (12)—these commitments have rarely been met.

Other barriers identified by researchers included poor government and institutional policies to support commercialization, inadequate or poorly understood intellectual property regimes, regulatory bureaucracy, and user perception favoring foreign products.

Commercializing Stagnant Technologies

Other than the noncorrupt exploitation of natural resources, turning creative ideas into goods and services that meet market needs is the main way countries develop. It happened in South Korea; is happening in India, China, and Brazil; and will happen in Africa. Unlike in developed countries, product development and commercialization in Africa at present primarily involves local improvements of technologies developed elsewhere, modifying imported technologies to suit local conditions (usually focused on cost reduction); development of novel products is less common (13). Although commercialization challenges remain in countries like China, India, and Brazil, the situation there is improving (14–16), and those countries’ products will provide stiff competition for African products. The immediate objective of product commercialization in Africa is not to replace or compete with globally known effective technologies, but to increase their accessibility through adaptation and cost reduction. But in the long term, once the commercialization culture has been embedded, it is hoped that Africa will compete more effectively with the rest of the world.

Commercialization culture has been embedded, it is hoped that Africa will compete more effectively with the rest of the world.

In every country, there are people claiming to have developed remedies or technologies, but, when subjected to rigorous testing and evaluation in clinical trials, many do not work. Some people believe the immediate priority of developing countries should be infrastructure and expertise development to rapidly field-test technologies to determine which ones are worthy of commercialization (17). We believe, however, that both validation and more-downstream commercialization activities—including regulation to ensure bad technologies are kept off the market—should be pursued in parallel to link innovation to the market and consumer needs.

Several health product development and commercialization initiatives are under way in Africa: the African Network for Drugs and Diagnostics Innovation, which aims to strengthen intra-African research collaboration (18); product development public-private partnerships, for example, one that led to a meningitis vaccine (19); and the U.S. President’s Emergency Plan for AIDS Relief investment model of enabling its contractors in Africa to attract private capital. However, none of these is focused on the pipeline of stagnant technologies.

From our previous work on technology development in Kenya, Uganda, Rwanda, Ghana, and Tanzania, three important needs emerged: proof-of-concept funds, networks linking scientists and entrepreneurs, and physical centers providing shared research infrastructure (20). Initiatives proposed to address these needs and to promote product commercialization in Africa include the Life Sciences Convergence Innovation Centers (21). These centers would bring together science, business, and capital and serve as “one-stop shops” for private investors who wish to tap into the pipeline of African technologies and to support an entrepreneurial approach to innovation in Africa.

Other than Bioventures, a venture fund in South Africa, we are unaware of any functioning VC fund in life sciences and health in sub-Saharan Africa. What is clearly needed is a VC fund to provide capital to promising ideas that are currently stagnating in research institutions. The fund could be shaped by lessons from venture funds in India (APIDC), China (BioVeda China), and South Africa (Bioventures), which include learning how to define the right mix between for-profit investments and social impact.

A sub-Saharan African VC fund for science-based health innovation could make

a huge difference, not only because of the money but also the mentorship and management experience that is brought to bear through the investments. An Innovation Center–VC fund platform could also be applied to other innovative sectors. The next big step in global health is to unlock this potential to create new opportunities to provide a pathway to prosperity, jobs, and better health.

References and Notes

1. A. S. Daar, E. M. Scrimgeour, in *Oxford Textbook of Surgery*, P. J. Morris and W. C. Wood, Eds. (Oxford Univ. Press, Oxford, 2000), pp. 3278–3292.
2. P. J. Hotez, A. Fenwick, *PLoS Negl. Trop. Dis.* **3**, e485 (2009).
3. Z. A. Andrade, *Parasite Immunol.* **31**, 656 (2009).
4. K. M. Bosompem, O. Owusu, E. O. Okanla, S. Kojima, *Trop. Med. Int. Health* **9**, 991 (2004).
5. J. Gans, S. Stern, *Res. Policy* **32**, 333 (2003).
6. South Africa was excluded from this study because it is classified as an emerging economy with regard to science and technology, and its inclusion would have skewed the findings. Kenya, which has the highest rate of patenting in all of Africa (outside South Africa), and Nigeria, sub-Saharan’s largest economy, were chosen because of their relatively well developed innovative sectors, including excellent scientific research institutions and financial sectors. Ghana, Tanzania, and Uganda were chosen because they represent middle-level African countries in this respect; Rwanda was chosen as a representative of the least-developed countries in both the innovative sector and scientific institutions. Northern African countries were excluded because they are not classified as part of sub-Saharan Africa (22).
7. B. Oyelaran-Oyeyinka, paper presented at *Innovation in the African Context: A Forum for Policymakers*, 6 to 8 March 2007, Dublin, Ireland.
8. F. C. Mills-Robertson *et al.*, *Pharm. Pharmacol.* **3**, 476 (2009).
9. P. A. Singer *et al.*, *Nature* **449**, 160 (2007).
10. P. Wamboga-Mugirya, *SciDevNet News*, 1 June 2006; www.scidev.net/en/news/us30m-millennium-science-initiative-for-uganda.html.
11. K. Chege, *SciDevNet News*, 20 June 2007; www.scidev.net/en/news/kenya-kick-starts-innovation-fund.html.
12. African Union commits to science in Addis Ababa Declaration, www.unesco.org/science/psd/thm_innov/addis_en.shtml.
13. J. E. Aubert, *Promoting Innovation in Developing Countries: A Conceptual Framework* (World Bank, Washington, DC, 2005).
14. S. E. Frew *et al.*, *Nat. Biotechnol.* **25**, 403 (2007).
15. S. E. Frew *et al.*, *Nat. Biotechnol.* **26**, 37 (2008).
16. R. Rezaie *et al.*, *Nat. Biotechnol.* **26**, 627 (2008).
17. C. Scott, *Nat. Med.* **13**, 228 (2007).
18. S. Nwaka *et al.*, *PLoS Med.* **7**, e1000293 (2010).
19. P. J. Hotez, A. S. Brown, *Biologicals* **37**, 160 (2009).
20. P. Singer *et al.*, in *Global Forum Update on Research for Health*, vol. 5, *Fostering Innovation for Global Health* (Global Forum for Health, Geneva, 2008), pp. 143–150.
21. H. Masum, A. S. Daar, S. Al-Bader, R. Shah, P. A. Singer, *Innovations* **2**, 129 (2007).
22. World Economic Forum (WEF), *Assessing Africa’s Competitiveness in a Global Context* (WEF, Geneva, 2007), chap. 1.1, pp. 3–7; www.weforum.org/pdf/gcr/africa/1.1.pdf.
23. This study was funded by grants from Genome Canada, Ontario Research Fund, Canadian Institutes of Health Research, and the Bill & Melinda Gates Foundation. P.A.S. is chief executive officer, Grand Challenges Canada; a paid adviser to BioVeda China and formerly a paid adviser to Merck Frosst; and a consultant to PepsiCo, Inc. The authors thank J. Clark for editorial comments.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1483/DC1

10.1126/science.1195401

MATERIALS SCIENCE

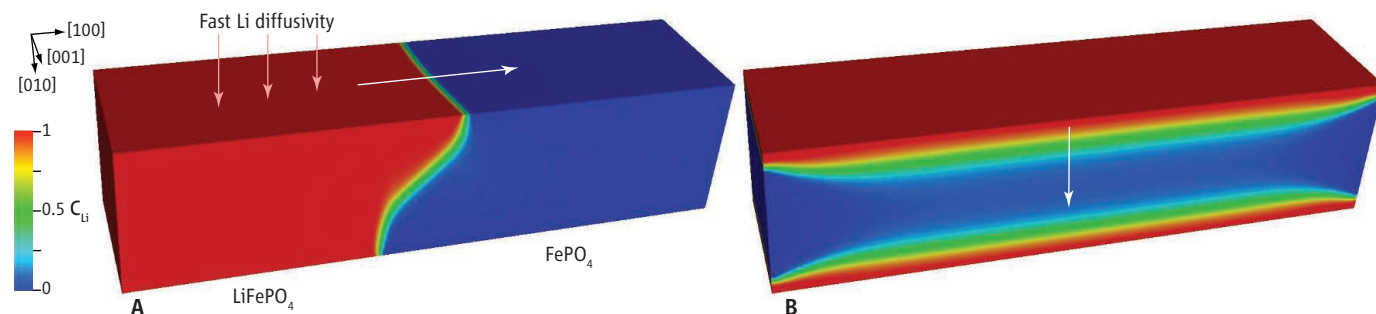
Building a Better Battery

Yet-Ming Chiang

Innovations in the battery field are infrequent and hard-won. New electrochemical systems (a new positive or negative electrode, electrolyte, or combination thereof) reach the marketplace only once every few years, and the energy density of lithium-ion batteries as a class has increased on average by only 8 to 9% per year since the early 1990s. Thus, in the burgeoning field of nanoscale electrode materials, skepticism regarding new claims is perhaps not surprising because of the many requirements that any battery electrode must simultaneously meet to be commercialized. One route by which battery performance can be com-

cerns specific to nanomaterials: Nanoparticle electrodes may have low packing density that makes high bulk energy density difficult to realize; synthesis methods may not be scalable and/or economical in a field where cost is a most important metric (e.g., electric vehicle and grid storage batteries); and nanoscale forms of inherently reactive compounds raise questions of safety and stability. There are also fundamental scientific questions centered on the mechanisms of electrochemical storage because of departures from known scaling laws as the size scale decreases. All of these issues need to be convincingly and simultaneously addressed if laboratory-based

Controlling the charge-induced morphological changes of electrode materials may provide a route to improved battery performance.



promised is by mechanical failure due to the large volume changes associated with the charge-discharge cycle. On page 1515 of this issue, Huang *et al.* (1) report an ingenious in situ transmission electron microscope (TEM) experiment that uses a low-vapor pressure ionic liquid electrolyte to allow imaging of a SnO_2 nanowire electrode in an “open” electrochemical cell. They observe a reaction mechanism in the SnO_2 nanowires that progresses sequentially along the nanowire from end to end, allowing them to accommodate a ~250% volume change without fracturing and at practical charging rates. These intriguing results raise the question of whether such one-dimensional phase transformations can be induced in other materials.

Nanomaterials offer the promise of high storage capacity at useful current rates, along with reversible cycling over hundreds to thousands of cycles and over a wide temperature range. However, there are practical con-

results are to be successfully translated into substantial progress in battery performance.

SnO_2 is a model for a class of metal oxides and fluorides (2–4) in which reversible storage capacities exceeding those possible with intercalation reactions are realized with displacement or “conversion” reactions that produce one or more new phases. Electrochemical reduction of the starting oxide by lithium produces Li_2O [LiF in the case of the fluorides (4)] and the parent metal, but unlike primary battery systems such as Li/MnO_2 that undergo the same reaction but are poorly reversible, reversible storage has been obtained when the product phases are nanoscale and reactive. In the case of SnO_2 , the Li_2O reaction product does not participate in reversible storage; subsequent cycling stores lithium through an alloying reaction with the nanoscale particles of metallic Sn. This was first established in SnO_2 -bearing glasses (5) and is also the case for Huang *et al.*’s nanowires. Thus, this reaction mechanism has the drawback of a large and irreversible loss of working lithium in the first cycle, whereas in other systems the conversion reaction produces nanoscale

Li_2O or LiF that does react reversibly with the metal electrode and is the main contributor to reversible capacity (2, 4). However, the large strain accommodation capability shown by the SnO_2 nanowires would be valuable if achievable in other high-capacity, high-strain electrodes such as silicon. Alloying with Li to the limiting composition $\text{Li}_{4.4}\text{Si}$ yields theoretically an enormous capacity of 4200 milliamperes hours per gram (mAh/g), versus 150 mAh/g for LiCoO_2 cathodes and 350 mAh/g for graphite anodes used in conventional lithium-ion batteries, but also produces a molar volume change of 311%. Silicon undergoes solid-state amorphization during lithiation

Coping with strain. (A) Phase field modeling reveals that under a moderate overpotential of 25 mV, misfit stress causes FePO_4 to grow along the [100] longitudinal direction, normal to the fast-diffusion direction. (B) Increasing the overpotential to 100 mV allows Li diffusion anisotropy to dominate the transformation morphology; diffuse phase boundaries migrate inward along the [010] direction. C_{Li} , lithium concentration

(6), and one strategy for strain accommodation is to limit the extent of lithiation (which sacrifices charge storage capacity) to maintain an amorphous phase (7). Silicon nanowires, on the other hand, have exhibited reversible capacities exceeding 3000 mAh/g (8).

In considering how one-dimensional transformation morphologies might be induced, an example from the first successfully commercialized nanoscale cathodes, the phospho-olivines, may be instructive. Strain accommodation (9, 10) and related phase transformation kinetics (11) are tunable features of nanoscale olivines and have been implicated as design criteria for high-power and long cycle life. The figure shows results using a phase-field model (12) to simulate possible transformation morphol-

ogies in a LiFePO_4 nanorod during the first-order phase transition to FePO_4 . The anisotropy of Li diffusion and the magnitude of misfit strain ($\sim 7\%$ volume strain) have been explicitly included, and the driving force for the transformation is varied via the electrical overpotential (13). At a moderate overpotential of 25 mV (see the figure, panel A), stress relaxation causes FePO_4 to grow along the [100] longitudinal direction, analogous to that of Huang *et al.*, even though Li diffusion is fastest normal to this direction. However, at a higher overpotential of 100 mV, the influence of strain energy is overcome and lateral Li diffusion dominates the transformation morphology (see the figure, panel B).

Thus, interactions among stress, transport anisotropy, and the magnitude of the driving force (among other variables) may influence

phase transformation morphology in nanowire electrodes. Other unanswered questions include the effect of electrolyte distribution: Is the one-dimensional transformation in SnO_2 facilitated by having a thin wetted layer of electrolyte, and would results differ for a “flooded” electrolyte battery? And could a competing radial reaction morphology also preserve the nanowire morphology? Regardless, the results presented by Huang *et al.* are testimony to the power of direct observation in electrochemical materials science, and they illustrate a previously unrecognized mode of reaction in battery electrodes. The results should stimulate others to consider analogous experiments and mechanisms in other storage materials, and should contribute to the design of nanoscale electrodes that fully exploit the potential of ultrahigh-capacity storage materials.

References

1. J. Y. Huang *et al.*, *Science* **330**, 1515 (2010).
2. P. Poizot, S. Laruelle, S. Grugeon, L. Dupont, J.-M. Tarascon, *Nature* **407**, 496 (2000).
3. H. Li, P. Balaya, J. Maier, *J. Electrochem. Soc.* **151**, A1878 (2004).
4. G. G. Amatucci, N. Pereira, *J. Fluor. Chem.* **128**, 243 (2007).
5. Y. Idota, T. Kubota, A. Matsufuji, Y. Maekawa, T. Miyasaka, *Science* **276**, 1395 (1997).
6. P. Limthongkul, Y.-I. Jang, N. J. Dudney, Y.-M. Chiang, *Acta Mater.* **51**, 1103 (2003).
7. M. N. Obrovac, L. J. Krause, *J. Electrochem. Soc.* **154**, A103 (2007).
8. C. K. Chan *et al.*, *Nat. Nanotechnol.* **3**, 31 (2008).
9. G. Chen, X. Song, T. Richardson, *Electrochem. Solid-State Lett.* **9**, A295 (2006).
10. N. Meethong, H.-Y. S. Huang, S. A. Speakman, W. C. Carter, Y.-M. Chiang, *Adv. Funct. Mater.* **17**, 1115 (2007).
11. N. Meethong, Y.-H. Kao, W. C. Carter, Y.-M. Chiang, *Chem. Mater.* **22**, 1088 (2010).
12. M. Tang, W. C. Carter, Y.-M. Chiang, *Annu. Rev. Mater. Res.* **40**, 501 (2010).
13. Y.-H. Kao *et al.*, *Chem. Mater.* **22**, 5845 (2010).

10.1126/science.1198591

PLANT SCIENCE

Genome Evolution in Plant Pathogens

Peter N. Dodds

Food security is of global importance and crop diseases caused by plant pathogens are a major constraint to agriculture worldwide. Many of these pathogens have a similar biotrophic life stage during which they contact host cells and secrete effector proteins that alter plant responses to infection (1). In this issue, comparative genomics studies of closely related pathogen species by Raffaele *et al.* on page 1540 (2), Baxter *et al.* on page 1549 (3), Spanu *et al.* on page 1543 (4), and Schirawski *et al.* on page 1546 (5) reveal that such effector proteins evolve rapidly and that their diversity contributes to host range and parasite speciation.

Biotrophic infection strategies have evolved independently in diverse lineages of plant pathogens. These include fungus-like parasites (oomycetes) from the kingdom Stramenopila, such as the destructive potato blight pathogen *Phytophthora infestans* (agent of the Irish potato famine), fungi such as powdery mildews (ascomycetes), and rust and smut fungi (basidiomycetes). These pathogens form specialized hyphae (called haustoria) that penetrate the plant cell wall



Agricultural threats. Many pathogens, such as *Phytophthora infestans*, target major food crops, such as potato (shown). The genes involved in host-pathogen interactions are highly diversified among related *Phytophthora* species that attack different plants.

and allow nutrient uptake from host tissue (6). These structures also secrete large repertoires of effector proteins that enter host cells and manipulate defense responses and cellular metabolism. Many oomycete effectors require the short amino acid motif RxLR (Arg, any amino acid, Leu, Arg) for entry into plant cells (7), independently of other pathogen machinery (8, 9). Some fungal effectors also enter host plant cells (10, 11), although they lack clearly conserved peptide motifs.

Raffaele *et al.* compared the genomes of four very closely related *Phytophthora* species that infect quite different host plant species (see the figure). The evolution of these

Pathogen genes that shut down specific host plant immune responses are highly divergent and have evolved rapidly to accommodate adaptation.

pathogens therefore involved relatively recent shifts in host range, followed by specialization to the new hosts. They found most pathogen genes and genome regions to be highly conserved, but genes involved in host-pathogen interaction appear highly diversified, especially the predicted RxLR-containing effectors. Most of these genes are located in gene-sparse, transposon-rich genome regions, suggesting that these features allow rapid evolution of effector loci after host changes. Genes involved in chromatin modification are also located in these regions and show extensive variation, suggesting that epigenetic regulation of gene expression also contributes to adaptation following host shifts.

Despite having a biotrophic life stage, *Phytophthora* species subsequently kill the infected parts of the plant but continue to feed on the dead plant tissue (and can be cultured on simple medium). By contrast, the related oomycete *Hyaloperonospora arabidopsidis*, which is a pathogen of the model plant *Arabidopsis thaliana*, is exclusively biotrophic and cannot be grown in culture. This pathogen is believed to have evolved from a *Phytophthora*-like hemibiotrophic ancestor. Baxter *et al.* found that its genome contains a unique set of diversified RxLR-containing effectors but has lost many of the hydrolytic

Division of Plant Industry, Commonwealth Scientific and Industrial Research Organisation, Canberra, ACT 2614, Australia. E-mail: peter.dodds@csiro.au

ogies in a LiFePO_4 nanorod during the first-order phase transition to FePO_4 . The anisotropy of Li diffusion and the magnitude of misfit strain ($\sim 7\%$ volume strain) have been explicitly included, and the driving force for the transformation is varied via the electrical overpotential (13). At a moderate overpotential of 25 mV (see the figure, panel A), stress relaxation causes FePO_4 to grow along the [100] longitudinal direction, analogous to that of Huang *et al.*, even though Li diffusion is fastest normal to this direction. However, at a higher overpotential of 100 mV, the influence of strain energy is overcome and lateral Li diffusion dominates the transformation morphology (see the figure, panel B).

Thus, interactions among stress, transport anisotropy, and the magnitude of the driving force (among other variables) may influence

phase transformation morphology in nanowire electrodes. Other unanswered questions include the effect of electrolyte distribution: Is the one-dimensional transformation in SnO_2 facilitated by having a thin wetted layer of electrolyte, and would results differ for a “flooded” electrolyte battery? And could a competing radial reaction morphology also preserve the nanowire morphology? Regardless, the results presented by Huang *et al.* are testimony to the power of direct observation in electrochemical materials science, and they illustrate a previously unrecognized mode of reaction in battery electrodes. The results should stimulate others to consider analogous experiments and mechanisms in other storage materials, and should contribute to the design of nanoscale electrodes that fully exploit the potential of ultrahigh-capacity storage materials.

References

1. J. Y. Huang *et al.*, *Science* **330**, 1515 (2010).
2. P. Poizot, S. Laruelle, S. Grugeon, L. Dupont, J.-M. Tarascon, *Nature* **407**, 496 (2000).
3. H. Li, P. Balaya, J. Maier, *J. Electrochem. Soc.* **151**, A1878 (2004).
4. G. G. Amatucci, N. Pereira, *J. Fluor. Chem.* **128**, 243 (2007).
5. Y. Idota, T. Kubota, A. Matsufuji, Y. Maekawa, T. Miyasaka, *Science* **276**, 1395 (1997).
6. P. Limthongkul, Y.-I. Jang, N. J. Dudney, Y.-M. Chiang, *Acta Mater.* **51**, 1103 (2003).
7. M. N. Obrovac, L. J. Krause, *J. Electrochem. Soc.* **154**, A103 (2007).
8. C. K. Chan *et al.*, *Nat. Nanotechnol.* **3**, 31 (2008).
9. G. Chen, X. Song, T. Richardson, *Electrochem. Solid-State Lett.* **9**, A295 (2006).
10. N. Meethong, H.-Y. S. Huang, S. A. Speakman, W. C. Carter, Y.-M. Chiang, *Adv. Funct. Mater.* **17**, 1115 (2007).
11. N. Meethong, Y.-H. Kao, W. C. Carter, Y.-M. Chiang, *Chem. Mater.* **22**, 1088 (2010).
12. M. Tang, W. C. Carter, Y.-M. Chiang, *Annu. Rev. Mater. Res.* **40**, 501 (2010).
13. Y.-H. Kao *et al.*, *Chem. Mater.* **22**, 5845 (2010).

10.1126/science.1198591

PLANT SCIENCE

Genome Evolution in Plant Pathogens

Peter N. Dodds

Food security is of global importance and crop diseases caused by plant pathogens are a major constraint to agriculture worldwide. Many of these pathogens have a similar biotrophic life stage during which they contact host cells and secrete effector proteins that alter plant responses to infection (1). In this issue, comparative genomics studies of closely related pathogen species by Raffaele *et al.* on page 1540 (2), Baxter *et al.* on page 1549 (3), Spanu *et al.* on page 1543 (4), and Schirawski *et al.* on page 1546 (5) reveal that such effector proteins evolve rapidly and that their diversity contributes to host range and parasite speciation.

Biotrophic infection strategies have evolved independently in diverse lineages of plant pathogens. These include fungus-like parasites (oomycetes) from the kingdom Stramenopila, such as the destructive potato blight pathogen *Phytophthora infestans* (agent of the Irish potato famine), fungi such as powdery mildews (ascomycetes), and rust and smut fungi (basidiomycetes). These pathogens form specialized hyphae (called haustoria) that penetrate the plant cell wall



Agricultural threats. Many pathogens, such as *Phytophthora infestans*, target major food crops, such as potato (shown). The genes involved in host-pathogen interactions are highly diversified among related *Phytophthora* species that attack different plants.

and allow nutrient uptake from host tissue (6). These structures also secrete large repertoires of effector proteins that enter host cells and manipulate defense responses and cellular metabolism. Many oomycete effectors require the short amino acid motif RxLR (Arg, any amino acid, Leu, Arg) for entry into plant cells (7), independently of other pathogen machinery (8, 9). Some fungal effectors also enter host plant cells (10, 11), although they lack clearly conserved peptide motifs.

Raffaele *et al.* compared the genomes of four very closely related *Phytophthora* species that infect quite different host plant species (see the figure). The evolution of these

Pathogen genes that shut down specific host plant immune responses are highly divergent and have evolved rapidly to accommodate adaptation.

pathogens therefore involved relatively recent shifts in host range, followed by specialization to the new hosts. They found most pathogen genes and genome regions to be highly conserved, but genes involved in host-pathogen interaction appear highly diversified, especially the predicted RxLR-containing effectors. Most of these genes are located in gene-sparse, transposon-rich genome regions, suggesting that these features allow rapid evolution of effector loci after host changes. Genes involved in chromatin modification are also located in these regions and show extensive variation, suggesting that epigenetic regulation of gene expression also contributes to adaptation following host shifts.

Despite having a biotrophic life stage, *Phytophthora* species subsequently kill the infected parts of the plant but continue to feed on the dead plant tissue (and can be cultured on simple medium). By contrast, the related oomycete *Hyaloperonospora arabidopsidis*, which is a pathogen of the model plant *Arabidopsis thaliana*, is exclusively biotrophic and cannot be grown in culture. This pathogen is believed to have evolved from a *Phytophthora*-like hemibiotrophic ancestor. Baxter *et al.* found that its genome contains a unique set of diversified RxLR-containing effectors but has lost many of the hydrolytic

Division of Plant Industry, Commonwealth Scientific and Industrial Research Organisation, Canberra, ACT 2614, Australia. E-mail: peter.dodds@csiro.au

enzymes that *Phytophthora* species use to digest host cell walls, as well as many of the genes that induce host cell death. The reduction in these protein classes is inferred as resulting from selection for “stealth,” allowing *H. arabidopsidis* to avoid triggering host defense responses during its extended biotrophic interaction. It is also deficient in metabolic processes that are shared by free-living organisms, such as the lack of nitrate and sulfate assimilation enzymes. Growing exclusively in living plant leaves, *H. arabidopsidis* relies on access to reduced nitrogen and sulfur from host cells.

Likewise, Spanu *et al.* found that the genomes of three species of fungal powdery mildew pathogens (also obligate biotrophs) are deficient in several classes of conserved primary and secondary metabolism genes. These include the nitrate and sulfate assimilation pathways and plant cell wall hydrolytic enzymes, suggesting that the independent evolution to obligate biotrophy that has occurred in the fungal and oomycete lineages involved convergent adaptation to specialize in the exclusively parasitic life-style in plant leaves. The metabolic deficiencies may provide clues for culturing these pathogens in vitro, which so far has proved difficult and hampered research on these organisms. The powdery mildew genomes do not encode RxLR-containing effectors, but encode a

unique class of secreted proteins with another conserved amino acid motif, YxC (Tyr, any amino acid, Cys). These genes are highly diverse among the three species, which infect very different host plants, suggesting that most of these effectors are associated with host species-specific adaptation.

In an intriguing twist on these studies, Schirawski *et al.* compared the genome of *Sporisorium reilianum* to that of the related fungal pathogen, *Ustilago maydis* (12), both of which infect maize. Most of the predicted secreted effectors are common to both species, but show much higher divergence than the rest of the genome. Thus, even within a host species, selection imposed by the host immune system or selection that targets different host processes can lead to rapid diversification of pathogen effectors. Most effector-encoding genes are located in small clusters in the genomes of the *U. maydis* and *S. reilianum*, and mutational analysis of these clusters has confirmed their important roles in infection (5, 12).

These studies highlight the value of comparative genomics in identifying important virulence genes with host-specific functions. The challenge now is to determine how the effector proteins that these genes encode turn the host cell to their own purposes. What are the specific targets of the effectors in the host and how do they contrib-

ute to disease pathogenesis? Understanding the molecular details of the biotrophic life-style and plant-microbe coevolution should lead to innovative disease control measures. Some effectors are recognized by components of the plant immune system, a characteristic that is exploited in plant breeding to improve disease resistance of crops. Thus, identifying effectors with important and nonredundant virulence functions that constrain their evolution may allow deployment of more durable resistance sources to safeguard world food production. The recent devastating impact of a new stem rust strain on wheat production in Africa (13) emphasizes the critical importance of developing lasting effective strategies to protect agricultural production from disease threats.

References

1. R. Panstruga, P. N. Dodds, *Science* **324**, 748 (2009).
2. S. Raffaele *et al.*, *Science* **330**, 1540 (2010).
3. L. Baxter *et al.*, *Science* **330**, 1549 (2010).
4. P. D. Spanu *et al.*, *Science* **330**, 1543 (2010).
5. J. Schirawski *et al.*, *Science* **330**, 1546 (2010).
6. R. T. Voegele, K. Mendgen, *New Phytol.* **159**, 93 (2003).
7. S. C. Whisson *et al.*, *Nature* **450**, 115 (2007).
8. D. Dou *et al.*, *Plant Cell* **20**, 1930 (2008).
9. S. D. Kale *et al.*, *Cell* **142**, 284 (2010).
10. M. Rafiqi *et al.*, *Plant Cell* **22**, 2017 (2010).
11. C. H. Khang *et al.*, *Plant Cell* **22**, 1388 (2010).
12. J. Kämper *et al.*, *Nature* **444**, 97 (2006).
13. R. P. Singh *et al.*, *Adv. Agron.* **98**, 271 (2008).

10.1126/science.1200245

MICROBIOLOGY

Sex and Sacrifice

Richard H. Kessin

The soil amoeba *Dictyostelium discoideum*, commonly known as a slime mold, has an asexual reproduction cycle that has made it a well-studied model organism. When starved, it creates streams of cells that use chemical cues (chemotaxis) to form aggregates, then migrating “slugs,” and finally spore-bearing fruiting bodies. The amoeba also has a less studied sexual cycle and is known for having three sexes, known as mating types I, II, and III. On page 1533 of this issue, Bloomfield *et al.* (1) put the sexual part of the slime mold’s life cycle on a solid molecular footing.

Why is the sexual cycle of a slime mold of interest at all? The answer, in part, involves the macrocyst, a remarkable structure formed

by sexual amoebae that has become an object of frustration and fascination for biologists. Consider this: Two haploid amoebae in the soil meet, and if they have compatible mating types, they fuse (2) (see the figure). The resulting zygote forms a so-called giant cell. It begins to eat up amoebae in its neighborhood, secreting cyclic adenosine monophosphate (cAMP), which attracts the chemotactic amoebae, siren-like, to their doom (3). (This is the same mechanism used to aggregate cells during fruiting body construction.) The chemotactic cells form an aggregate around the giant cell and synthesize a primary cell wall. The giant cell in the center of the aggregate gradually chews its way to the periphery, killing and consuming hundreds of amoebae (4). It is one of the great acts of phagocytosis in biology. The resulting structure—the macrocyst—is now 500 to 1000 times the size of a single cell and has three cellulose walls.

A single genetic locus determines the three sexes of slime mold amoebae.

The genetics underlying macrocyst formation, however, were poorly understood. Researchers had problems germinating macrocysts in the laboratory and identifying useful recombinant strains. The strains used in earlier experiments were separate wild isolates and may have evolved incompatibility mechanisms. In contrast, Bloomfield *et al.* produced strains that are genetically identical except at the mating-type locus, which may help in meiotic studies.

Bloomfield *et al.* reasoned that they could locate the mating-type locus of *D. discoideum* by searching for genes present in one mating type but absent (or highly diverged) in the others. They were right. Using DNA microarrays to analyze 10 wild strains, they identified a locus called *matA* that is present in all type I strains and has the coding capacity to make a small protein that would lack homology to any known protein. It has no charac-

Department of Pathology and Cell Biology, College of Physicians and Surgeons of Columbia University, New York, NY 10032, USA. E-mail: rhk2@columbia.edu

enzymes that *Phytophthora* species use to digest host cell walls, as well as many of the genes that induce host cell death. The reduction in these protein classes is inferred as resulting from selection for “stealth,” allowing *H. arabidopsidis* to avoid triggering host defense responses during its extended biotrophic interaction. It is also deficient in metabolic processes that are shared by free-living organisms, such as the lack of nitrate and sulfate assimilation enzymes. Growing exclusively in living plant leaves, *H. arabidopsidis* relies on access to reduced nitrogen and sulfur from host cells.

Likewise, Spanu *et al.* found that the genomes of three species of fungal powdery mildew pathogens (also obligate biotrophs) are deficient in several classes of conserved primary and secondary metabolism genes. These include the nitrate and sulfate assimilation pathways and plant cell wall hydrolytic enzymes, suggesting that the independent evolution to obligate biotrophy that has occurred in the fungal and oomycete lineages involved convergent adaptation to specialize in the exclusively parasitic life-style in plant leaves. The metabolic deficiencies may provide clues for culturing these pathogens in vitro, which so far has proved difficult and hampered research on these organisms. The powdery mildew genomes do not encode RxLR-containing effectors, but encode a

unique class of secreted proteins with another conserved amino acid motif, YxC (Tyr, any amino acid, Cys). These genes are highly diverse among the three species, which infect very different host plants, suggesting that most of these effectors are associated with host species-specific adaptation.

In an intriguing twist on these studies, Schirawski *et al.* compared the genome of *Sporisorium reilianum* to that of the related fungal pathogen, *Ustilago maydis* (12), both of which infect maize. Most of the predicted secreted effectors are common to both species, but show much higher divergence than the rest of the genome. Thus, even within a host species, selection imposed by the host immune system or selection that targets different host processes can lead to rapid diversification of pathogen effectors. Most effector-encoding genes are located in small clusters in the genomes of the *U. maydis* and *S. reilianum*, and mutational analysis of these clusters has confirmed their important roles in infection (5, 12).

These studies highlight the value of comparative genomics in identifying important virulence genes with host-specific functions. The challenge now is to determine how the effector proteins that these genes encode turn the host cell to their own purposes. What are the specific targets of the effectors in the host and how do they contrib-

ute to disease pathogenesis? Understanding the molecular details of the biotrophic life-style and plant-microbe coevolution should lead to innovative disease control measures. Some effectors are recognized by components of the plant immune system, a characteristic that is exploited in plant breeding to improve disease resistance of crops. Thus, identifying effectors with important and nonredundant virulence functions that constrain their evolution may allow deployment of more durable resistance sources to safeguard world food production. The recent devastating impact of a new stem rust strain on wheat production in Africa (13) emphasizes the critical importance of developing lasting effective strategies to protect agricultural production from disease threats.

References

1. R. Panstruga, P. N. Dodds, *Science* **324**, 748 (2009).
2. S. Raffaele *et al.*, *Science* **330**, 1540 (2010).
3. L. Baxter *et al.*, *Science* **330**, 1549 (2010).
4. P. D. Spanu *et al.*, *Science* **330**, 1543 (2010).
5. J. Schirawski *et al.*, *Science* **330**, 1546 (2010).
6. R. T. Voegele, K. Mendgen, *New Phytol.* **159**, 93 (2003).
7. S. C. Whisson *et al.*, *Nature* **450**, 115 (2007).
8. D. Dou *et al.*, *Plant Cell* **20**, 1930 (2008).
9. S. D. Kale *et al.*, *Cell* **142**, 284 (2010).
10. M. Rafiqi *et al.*, *Plant Cell* **22**, 2017 (2010).
11. C. H. Khang *et al.*, *Plant Cell* **22**, 1388 (2010).
12. J. Kämper *et al.*, *Nature* **444**, 97 (2006).
13. R. P. Singh *et al.*, *Adv. Agron.* **98**, 271 (2008).

10.1126/science.1200245

MICROBIOLOGY

Sex and Sacrifice

Richard H. Kessin

The soil amoeba *Dictyostelium discoideum*, commonly known as a slime mold, has an asexual reproduction cycle that has made it a well-studied model organism. When starved, it creates streams of cells that use chemical cues (chemotaxis) to form aggregates, then migrating “slugs,” and finally spore-bearing fruiting bodies. The amoeba also has a less studied sexual cycle and is known for having three sexes, known as mating types I, II, and III. On page 1533 of this issue, Bloomfield *et al.* (1) put the sexual part of the slime mold’s life cycle on a solid molecular footing.

Why is the sexual cycle of a slime mold of interest at all? The answer, in part, involves the macrocyst, a remarkable structure formed

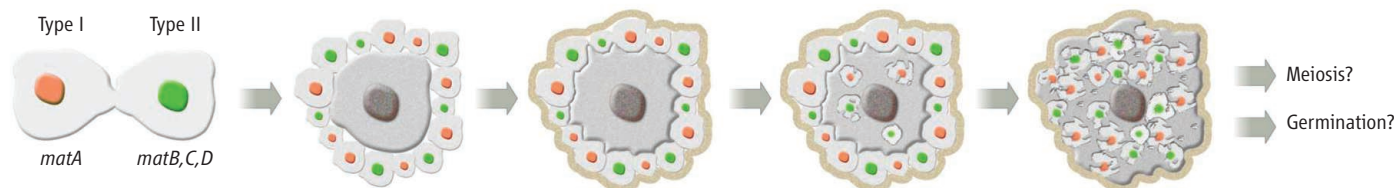
by sexual amoebae that has become an object of frustration and fascination for biologists. Consider this: Two haploid amoebae in the soil meet, and if they have compatible mating types, they fuse (2) (see the figure). The resulting zygote forms a so-called giant cell. It begins to eat up amoebae in its neighborhood, secreting cyclic adenosine monophosphate (cAMP), which attracts the chemotactic amoebae, siren-like, to their doom (3). (This is the same mechanism used to aggregate cells during fruiting body construction.) The chemotactic cells form an aggregate around the giant cell and synthesize a primary cell wall. The giant cell in the center of the aggregate gradually chews its way to the periphery, killing and consuming hundreds of amoebae (4). It is one of the great acts of phagocytosis in biology. The resulting structure—the macrocyst—is now 500 to 1000 times the size of a single cell and has three cellulose walls.

A single genetic locus determines the three sexes of slime mold amoebae.

The genetics underlying macrocyst formation, however, were poorly understood. Researchers had problems germinating macrocysts in the laboratory and identifying useful recombinant strains. The strains used in earlier experiments were separate wild isolates and may have evolved incompatibility mechanisms. In contrast, Bloomfield *et al.* produced strains that are genetically identical except at the mating-type locus, which may help in meiotic studies.

Bloomfield *et al.* reasoned that they could locate the mating-type locus of *D. discoideum* by searching for genes present in one mating type but absent (or highly diverged) in the others. They were right. Using DNA microarrays to analyze 10 wild strains, they identified a locus called *matA* that is present in all type I strains and has the coding capacity to make a small protein that would lack homology to any known protein. It has no charac-

Department of Pathology and Cell Biology, College of Physicians and Surgeons of Columbia University, New York, NY 10032, USA. E-mail: rhk2@columbia.edu



Macrocyst formation in *Dictyostelium*. Under certain culture conditions, haploid amoebae with different mating-type alleles (type I and type II shown) fuse to form a zygote called a giant cell. This cell ingests its neighbors and secretes cAMP to attract other amoebae into an aggregate with the giant cell inside. The aggregated cells secrete a primary wall while the giant cell gradually consumes them. Mature macrocysts have three cellulose walls (not shown) and can have a diameter of more than 100 μm .

teristic motifs—nothing to give a clue to its function, except for the fact that it is charged and probably is a cytosolic product. A strain carrying a deletion of *matA* is sterile and will not fuse with type II cells. Restore the gene and it will fuse.

What of the type II locus? Because the genes flanking the *matA* locus are present in strains of all mating types, the authors could recover the type II locus via the polymerase chain reaction (PCR). The type II locus contains three genes: *matB*, which is homologous to the type I *matA* but has diverged; *matC*, which has no homology to other known genes; and *matD*, which encodes a large secreted protein that may be anchored by glycosylphosphatidylinositol (GPI). Type II strains from all over the world have these three genes and they have hardly diverged at all. What happens when the three type II genes are put into the type I knockout? Happily, the amoeba switches to type II, which is to say it mates with type I strains to form macrocysts. The loci are sufficient to determine mating type.

There is a third mating type, type III, which is capable of mating with types I or II but not with itself. Rare homothallic strains also exist and the authors have looked at these as well. Type III strains contain two genes homologous to *matC* and *matD* of the type II strain, and these have been named *matS* and *matT*.

By transforming the *matA* null strain with individual genes, the authors have begun to ask which of these genes is essential for the formation of the macrocyst. Do these macrocysts have the capacity to carry out meiosis and provide researchers with a proper tetrad of recombinants? We will see. For *Dictyostelium*, the identification of such a capability, although arguably decades late for geneticists (more if you work on peas or *Drosophila*), would still be wonderfully useful. Sequencing of single-nucleotide poly-

morphisms has yielded evidence that wild strains have recombined frequently (5). There is a parasexual system of genetic analysis (which involves nonsexual mechanisms for transferring genetic material without meiosis), but it does not have the flexibility or power of meiotic genetics.

The enormous phagocytic capacity of the giant cell offers interesting problems. How do the haploid amoebae suddenly get defined as foreign and targeted for phagocytosis? How does the lysosomal system suddenly ramp up? Does the bacterium *Legionella pneumophila*, known to be capable of derailing phagosome-lysosome fusion in an amoeba (6), stand a chance in a giant cell? How does a genome designed for an amoeba handle a cell that is now 1000 times as large? Perhaps with these genes in hand, the formation of macrocysts can be synchronized and the essential genes identified. There is evidence that these genes will overlap with the genes that control fruiting body formation, for which the macrocyst is probably an evolutionary precursor (7).

The evolutionary biologists who have recently taken an interest in *Dictyostelium*

should prick up their ears. How does a system evolve in which more than 99% of cells perish, as opposed to 20% during the fruiting body version of development? Imagine a population of 999 type I amoebae and one type II amoeba—a single giant cell will form, and it will consume the 998 other amoebae. If the resulting macrocyst then germinates and produces meiotic offspring, the frequency of the type II gene will have gone from 0.001 to 0.5. There could be a balancing of mating types, or perhaps mutant amoebae would be selected that decline the chemotactic call of the giant cell. What was a fragmented literature has now gained a solid footing.

References

1. G. Bloomfield, J. Skelton, A. Ivens, Y. Tanaka, R. R. Kay, *Science* **330**, 1533 (2010).
2. J. C. Blaskovics, K. B. Raper, *Biol. Bull.* **113**, 58 (1957).
3. D. H. O'Day, *Can. J. Microbiol.* **25**, 1416 (1979).
4. M. A. Wallace, K. B. Raper, *J. Gen. Microbiol.* **113**, 327 (1979).
5. J. M. Flowers *et al.*, *PLoS Genet.* **6**, e1001013 (2010).
6. G. P. Otto *et al.*, *Mol. Microbiol.* **51**, 63 (2004).
7. R. H. Kessin, *Dictyostelium: Evolution, Cell Biology, and the Development of Multicellularity* (Cambridge Univ. Press, Cambridge, 2001).

10.1126/science.1199899

GENETICS

First-Class Control of HIV-1

Andrew J. McMichael¹ and E. Yvonne Jones²

Genome-wide association studies reveal amino acids of the major histocompatibility complex that associate with the rate of progression to AIDS.

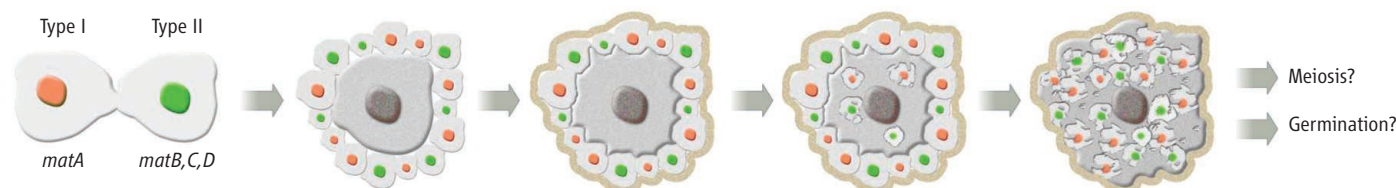
The role of infectious disease in driving human genetic variations (polymorphisms) was first clearly espoused by J. B. S. Haldane in 1949 (1). Once the major histocompatibility complex (MHC) was established as the most polymorphic mammalian genetic system, searches began for human MHC [or human leukocyte antigen (HLA)] associations with infectious

disease resistance. Such findings have been rare, though, possibly because susceptibility genes have been deselected over evolution. But the appearance of completely new infections caused by viruses, such as HIV-1, has opened opportunities to look at such selection in the MHC as it happens. On page 1551 of this issue, the International HIV Controllers Study (2), demonstrates the central role of HLA class I in controlling HIV-1 infection.

Earlier studies had demonstrated the influence of HLA type on slow versus rapid AIDS progression (3). Protection is thought to be mediated by strong T cell (CD8 subtype) responses to viral peptides presented

¹Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DS, UK.

²Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, UK. E-mail: andrew.mcmichael@ndm.ox.ac.uk; yvonne@strubi.ox.ac.uk



Macrocyst formation in *Dictyostelium*. Under certain culture conditions, haploid amoebae with different mating-type alleles (type I and type II shown) fuse to form a zygote called a giant cell. This cell ingests its neighbors and secretes cAMP to attract other amoebae into an aggregate with the giant cell inside. The aggregated cells secrete a primary wall while the giant cell gradually consumes them. Mature macrocysts have three cellulose walls (not shown) and can have a diameter of more than 100 μm .

teristic motifs—nothing to give a clue to its function, except for the fact that it is charged and probably is a cytosolic product. A strain carrying a deletion of *matA* is sterile and will not fuse with type II cells. Restore the gene and it will fuse.

What of the type II locus? Because the genes flanking the *matA* locus are present in strains of all mating types, the authors could recover the type II locus via the polymerase chain reaction (PCR). The type II locus contains three genes: *matB*, which is homologous to the type I *matA* but has diverged; *matC*, which has no homology to other known genes; and *matD*, which encodes a large secreted protein that may be anchored by glycosylphosphatidylinositol (GPI). Type II strains from all over the world have these three genes and they have hardly diverged at all. What happens when the three type II genes are put into the type I knockout? Happily, the amoeba switches to type II, which is to say it mates with type I strains to form macrocysts. The loci are sufficient to determine mating type.

There is a third mating type, type III, which is capable of mating with types I or II but not with itself. Rare homothallic strains also exist and the authors have looked at these as well. Type III strains contain two genes homologous to *matC* and *matD* of the type II strain, and these have been named *matS* and *matT*.

By transforming the *matA* null strain with individual genes, the authors have begun to ask which of these genes is essential for the formation of the macrocyst. Do these macrocysts have the capacity to carry out meiosis and provide researchers with a proper tetrad of recombinants? We will see. For *Dictyostelium*, the identification of such a capability, although arguably decades late for geneticists (more if you work on peas or *Drosophila*), would still be wonderfully useful. Sequencing of single-nucleotide poly-

morphisms has yielded evidence that wild strains have recombined frequently (5). There is a parasexual system of genetic analysis (which involves nonsexual mechanisms for transferring genetic material without meiosis), but it does not have the flexibility or power of meiotic genetics.

The enormous phagocytic capacity of the giant cell offers interesting problems. How do the haploid amoebae suddenly get defined as foreign and targeted for phagocytosis? How does the lysosomal system suddenly ramp up? Does the bacterium *Legionella pneumophila*, known to be capable of derailing phagosome-lysosome fusion in an amoeba (6), stand a chance in a giant cell? How does a genome designed for an amoeba handle a cell that is now 1000 times as large? Perhaps with these genes in hand, the formation of macrocysts can be synchronized and the essential genes identified. There is evidence that these genes will overlap with the genes that control fruiting body formation, for which the macrocyst is probably an evolutionary precursor (7).

The evolutionary biologists who have recently taken an interest in *Dictyostelium*

should prick up their ears. How does a system evolve in which more than 99% of cells perish, as opposed to 20% during the fruiting body version of development? Imagine a population of 999 type I amoebae and one type II amoeba—a single giant cell will form, and it will consume the 998 other amoebae. If the resulting macrocyst then germinates and produces meiotic offspring, the frequency of the type II gene will have gone from 0.001 to 0.5. There could be a balancing of mating types, or perhaps mutant amoebae would be selected that decline the chemotactic call of the giant cell. What was a fragmented literature has now gained a solid footing.

References

1. G. Bloomfield, J. Skelton, A. Ivens, Y. Tanaka, R. R. Kay, *Science* **330**, 1533 (2010).
2. J. C. Blaskovics, K. B. Raper, *Biol. Bull.* **113**, 58 (1957).
3. D. H. O'Day, *Can. J. Microbiol.* **25**, 1416 (1979).
4. M. A. Wallace, K. B. Raper, *J. Gen. Microbiol.* **113**, 327 (1979).
5. J. M. Flowers *et al.*, *PLoS Genet.* **6**, e1001013 (2010).
6. G. P. Otto *et al.*, *Mol. Microbiol.* **51**, 63 (2004).
7. R. H. Kessin, *Dictyostelium: Evolution, Cell Biology, and the Development of Multicellularity* (Cambridge Univ. Press, Cambridge, 2001).

10.1126/science.1199899

GENETICS

First-Class Control of HIV-1

Andrew J. McMichael¹ and E. Yvonne Jones²

Genome-wide association studies reveal amino acids of the major histocompatibility complex that associate with the rate of progression to AIDS.

The role of infectious disease in driving human genetic variations (polymorphisms) was first clearly espoused by J. B. S. Haldane in 1949 (1). Once the major histocompatibility complex (MHC) was established as the most polymorphic mammalian genetic system, searches began for human MHC [or human leukocyte antigen (HLA)] associations with infectious

disease resistance. Such findings have been rare, though, possibly because susceptibility genes have been deselected over evolution. But the appearance of completely new infections caused by viruses, such as HIV-1, has opened opportunities to look at such selection in the MHC as it happens. On page 1551 of this issue, the International HIV Controllers Study (2), demonstrates the central role of HLA class I in controlling HIV-1 infection.

Earlier studies had demonstrated the influence of HLA type on slow versus rapid AIDS progression (3). Protection is thought to be mediated by strong T cell (CD8 subtype) responses to viral peptides presented

¹Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DS, UK.

²Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, UK. E-mail: andrew.mcmichael@ndm.ox.ac.uk; yvonne@strubi.ox.ac.uk

by HLA molecules (4). Polymorphic killer inhibitory immunoglobulin-like receptors (KIRs) expressed by natural killer cells are also associated with good virus control, but only in the presence of the HLA molecules that they bind, which include those associated with slow AIDS progression (HLA types B*57 and B*27) (5, 6).

The first genome-wide association study (GWAS) of an infectious disease (7, 8) identified striking associations between three single-nucleotide polymorphisms (SNPs) and the low level of viremia (which predicts slower disease progression) in the asymptomatic stage of HIV-1 infection. All three SNPs mapped close to the highly polymorphic *HLA A*, *B*, and *C* class I loci. The strongest associations were in the *HCP5* gene near *HLA-B*, a marker for *HLA B**57, and in a noncoding site 35 kb upstream of *HLA-C*.

The International HIV Controllers Study explored the role of the *HLA* system in controlling HIV-1 infection in more detail. A large cohort of 3622 patients, untreated with antiretroviral drugs, was divided into those who controlled HIV-1 infection well and those who progressed more rapidly to AIDS. Patients of European ancestry showed associations with *HCP5* and *HLA-C*, SNPs previ-

ously associated with a low level of viremia (7, 8). The study then referred to an earlier GWAS of diabetes (9) in which 2767 HLA-typed individuals were mapped for a similar set of SNPs. By imputing the HLA type (10) in the HIV-1-infected cohort, the study found that HLA types B*57:01, B*27:05, and B*14 were protective, whereas types B*35 and C*07 were associated with progression to AIDS. These results confirm and extend previous HLA typing data in HIV-1 infection (3).

Examination of all the polymorphic amino acids in HLA class I revealed that amino acid positions 67, 70, and particularly 97, in the peptide binding groove of HLA class I (see the figure) had stronger statistical associations with HIV-1 protection than the whole HLA molecules. Valine, asparagine, and tryptophan occur at position 97 in the B*57:01, B*27:05, and B*14 protective haplotypes, respectively, whereas arginine occurs in risk HLA molecules B*35, B*53, and C*07. This implies that these residues influence control of HIV-1 infection independently of the rest of the HLA molecule.

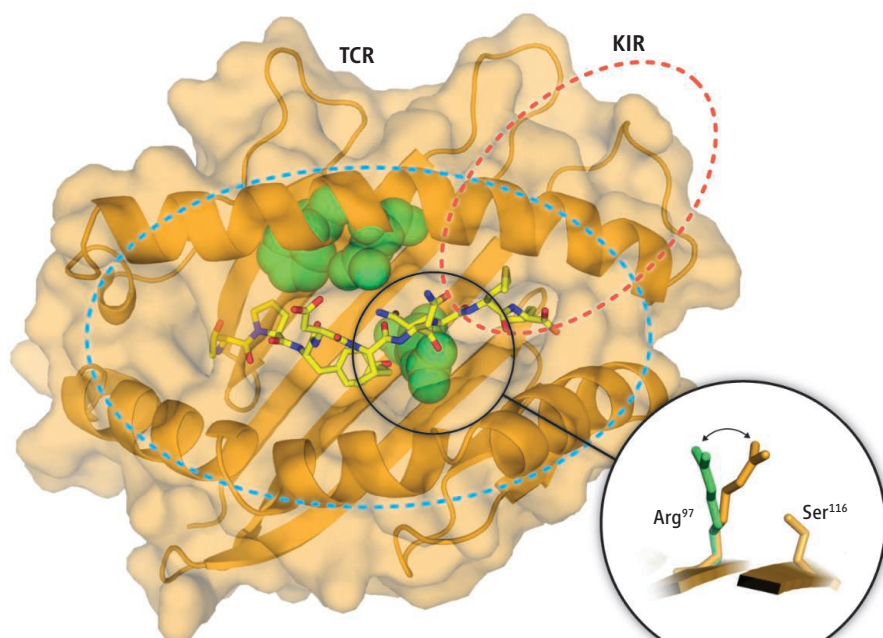
The HLA class I binding groove presents peptides of 8 to 10 amino acids with residue side chains at positions 2 and the carboxyl terminus serving as anchors, respec-

tively inserting into pockets B and F within the groove. These anchor points are usually supplemented by interactions in the center of the peptide and binding groove. The central-region interactions with different peptides vary greatly, resulting in very different peptide conformations. How might individual HLA residues within the binding groove confer the HIV protective effect?

Although residue 67 contributes to the specificity of the B pocket, none of the residues highlighted by the SNP associations influence the binding characteristics of the F pocket. Indeed, the gross peptide binding motif determined by the B and F pockets is rather similar for the protective B*57 and the nonprotective B*35; both methionine 67 (in B*57) and phenylalanine 67 (in B*35) are large hydrophobic side chains contributing to shallow B pockets that bind relatively small side chains at position 2, whereas the F pockets for both alleles bind aromatic residues at the carboxyl terminus. Residue 97 is centrally placed in the floor of the binding groove. Arginine is the most common residue at this position and present in the HLA molecules that confer risk for HIV-1 disease. It provides flexibility, in the presence of serine at position 116 (as in the risk HLA types), potentially allowing the binding groove to adapt to fit more peptides (11). This fits with the idea that protective HLA molecules bind fewer peptides, which delete fewer self-reactive T cells in the thymus, giving virus-specific T cells broader fine specificity (12). This could reduce virus options to escape by mutation (13, 14).

Whatever the mechanism, HIV-1 peptides bound to the HLA molecule are critical in protecting against AIDS. Much of the protective effect of B*57 is probably due to serendipitous binding of immunogenic peptides that come from the conserved HIV-1 Gag p24 protein. Gag forms the viral capsid structure, and mutations in this protein that are selected by T cells can weaken the virus (15). This could work in concert with the broader T cell specificity to limit escape.

Natural killer cells might also contribute to protection. They express the genetically polymorphic receptors KIR3DL1 and KIR3DS1, which bind to the peptide-HLA complex with a footprint that covers HLA B residues 77, 80, 81, and 84 as well as the carboxyl terminus of the bound peptide. Therefore, the HLA residues associated with effective control of HIV-1 might influence the action of natural killer cells. Weak genetic associations between HIV-1 control and the polymorphic residues 80, 77, and 81 may reflect the relative rarity of the relevant KIR3DL1 and



In the groove. The peptide binding groove of HLA B*53 is depicted as a main-chain ribbon (light brown) and transparent surface with side chains of residues 67, 70, and 97 highlighted in spheres (green). The viral peptide (shown in stick format) and the helices flanking the HLA groove contribute to the binding surfaces for T cell receptors (TCR) as well as the killer inhibitory immunoglobulin-like receptors (KIR) expressed by natural killer cells. (Inset) Arginine 97 is shown with rotated viewpoint to illustrate the conformational flexibility in risk molecules such as HLA B*53. The crystal structure of B*53 (Protein Data Bank deposition codes 1a1m and 1a1o) was drawn using PyMOL and Adobe Photoshop. The viral peptide sequence is TPYDINQML (threonine, proline, tyrosine, aspartic acid, isoleucine, asparagine, glutamine, methionine, leucine).

KIR3DS1 receptors in the population.

The two GWAS studies (2, 7, 8) demonstrate extreme dominance of the HLA class I region in the genetic association with control of HIV-1 infection. This accounts for nearly 20% of the observed variability in HIV-1 control. Given that both protective and highly susceptible alleles are quite rare, the importance of T cell and/or natural killer cell control is extraordinary and rewards massive efforts to determine the structure and function of these

genes and the molecules they encode. The question now is whether those not blessed with these protective genes can be immunologically pushed to make similar responses.

References

1. J. Lederberg, *Genetics* **153**, 1 (1999).
2. The International HIV Controllers Study, *Science* **330**, 1551 (2010); 10.1126/science.1195271.
3. M. Carrington, S. J. O'Brien, *Annu. Rev. Med.* **54**, 535 (2003).
4. H. Streeck *et al.*, *J. Virol.* **81**, 7725 (2007).
5. M. P. Martin *et al.*, *Nat. Genet.* **31**, 429 (2002).

6. M. P. Martin *et al.*, *Nat. Genet.* **39**, 733 (2007).
7. J. Fellay *et al.*, *PLoS Genet.* **5**, e1000791 (2009).
8. J. Fellay *et al.*, *Science* **317**, 944 (2007).
9. W. M. Brown *et al.*, *Diabetes Obes. Metab.* **11** (suppl. 1), 2 (2009).
10. S. Leslie, P. Donnelly, G. McVean, *Am. J. Hum. Genet.* **82**, 48 (2008).
11. K. J. Smith *et al.*, *Immunity* **4**, 215 (1996).
12. A. Kosmrlj *et al.*, *Nature* **465**, 350 (2010).
13. W. Fischer *et al.*, *PLoS ONE* **5**, e12303 (2010).
14. E. L. Turnbull *et al.*, *J. Immunol.* **176**, 6130 (2006).
15. J. Martinez-Picado *et al.*, *J. Virol.* **80**, 3617 (2006).

10.1126/science.1200035

GEOCHEMISTRY

Earth's Second Wind

Lee R. Kump

One of the great mysteries of Earth history is why, after 3 billion years of evolution, about 600 million years ago (Ma), organisms emerged from the microscopic world and shortly thereafter developed skeletons and shells (1). Two prevalent hypotheses include climate cooling (2) and an increase in the oxygen content of the ocean (3). To date, the best evidence in support of the oxygen theory has been the size of animals themselves: Larger animals have higher oxygen demands that can only be met by oxygen-rich waters. Now Dahl *et al.* (4) present independent proxy evidence for an increase of oceanic (and by inference, atmospheric) oxygen concentrations—from levels below the lower limit for large animals, to levels above—at about 550 Ma. This second rise of oxygen follows nearly 2 billion years after the well-documented first “Great Oxidation Event” (GOE) at about 2400 Ma.

The proxy used by these researchers is the isotopic composition of marine aqueous molybdenum (Mo), preserved in sedimentary rocks rich in organic matter and pyrite (an iron sulfide mineral) (see the figure, panel A). The ocean receives Mo from the weathering of rocks on land and deposits it in sediments. Under reducing conditions such as existed in soil environments before 2400 Ma, Mo is immobile.

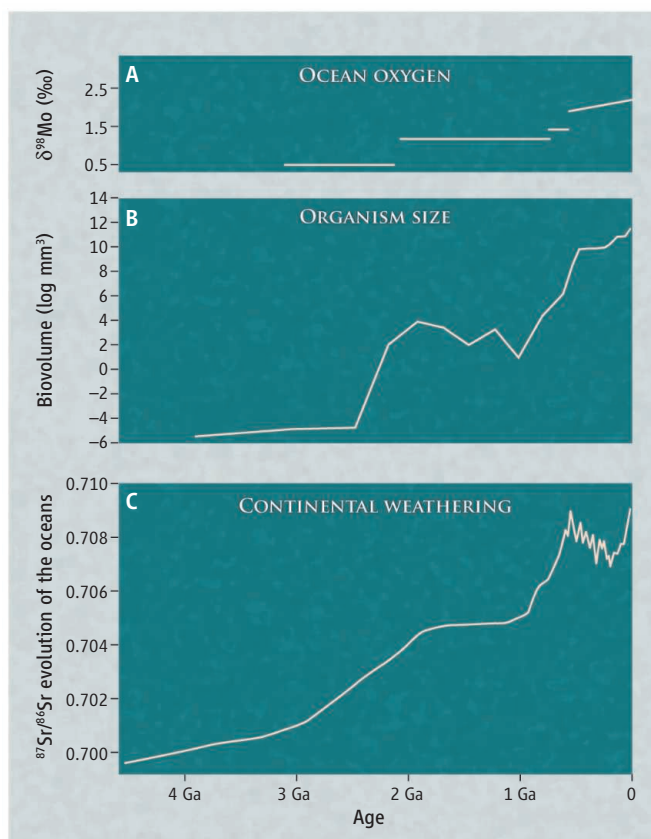
In the presence of oxygen, however, it forms the soluble molybdate ion and can be mobilized from soils into river waters and delivered to the ocean.

Although Mo accumulates in an oxygen-

Studies of molybdenum isotopes in sediments support the idea that increases in atmospheric oxygen in the Devonian period drove increases in animal size.

ated ocean, it ultimately is removed into sediments. These sedimentary sinks exist in well-oxygenated conditions, where Mo is removed with Fe and Mn oxides (the oxic sink), and in basins where reducing conditions prevail, and Mo is converted to thiomolybdate species that are scavenged into organic matter and sulfide minerals (the reducing sink). The oxic sink accumulates more of the lighter isotopes of Mo, whereas the reducing sink quantitatively removes the isotopes in their ambient proportions. When oxic sinks are scarce, the Mo isotopic composition of the ocean (and in reducing sediments) reflects the riverine input from recent continental weathering, whereas when oxic sinks are abundant (as they are today), the ocean's isotope ratio of heavy (^{98}Mo) to light (^{95}Mo) increases. Samples from the (relatively scarce) reducing sediments faithfully record the oxic ocean's “heavy” isotopic composition.

A compilation of existing analyses of Mo isotopic compositions of rocks of all ages (4) indicates low ratios until 2.7 billion years ago (Ga)—that is, prior to the GOE—when the ratio of $^{98}\text{Mo}/^{95}\text{Mo}$ increased from 0.7 (equivalent to the presumed riverine input) to 1.1 (5). This value was sustained through much of the Proterozoic (4) except for a dip between 2.4 and 2.2 Ga tied perhaps to glaciation (6). The isotope ratio shifted more posi-



Oxygen in the oceans and the atmosphere. Several proxies suggest an increase in atmospheric oxygen about 550 Ma. (A) An increase in the standardized $^{98}\text{Mo}/^{95}\text{Mo}$ ratio of the oceans through time reflects the increased oxygenation of the ocean (4). (B) The increase in the size of organisms recorded in the fossil record is shown (1). (C) The evolution of the Sr isotopic composition of the ocean (6) shows a stepwise increase in the $^{87}\text{Sr}/^{86}\text{Sr}$, which reflects the increasing influence of continental weathering over time on the composition of the ocean.

Department of Geosciences, Pennsylvania State University, University Park, PA 16802, USA. E-mail: lkump@psu.edu

KIR3DS1 receptors in the population.

The two GWAS studies (2, 7, 8) demonstrate extreme dominance of the HLA class I region in the genetic association with control of HIV-1 infection. This accounts for nearly 20% of the observed variability in HIV-1 control. Given that both protective and highly susceptible alleles are quite rare, the importance of T cell and/or natural killer cell control is extraordinary and rewards massive efforts to determine the structure and function of these

genes and the molecules they encode. The question now is whether those not blessed with these protective genes can be immunologically pushed to make similar responses.

References

1. J. Lederberg, *Genetics* **153**, 1 (1999).
2. The International HIV Controllers Study, *Science* **330**, 1551 (2010); 10.1126/science.1195271.
3. M. Carrington, S. J. O'Brien, *Annu. Rev. Med.* **54**, 535 (2003).
4. H. Streeck *et al.*, *J. Virol.* **81**, 7725 (2007).
5. M. P. Martin *et al.*, *Nat. Genet.* **31**, 429 (2002).

6. M. P. Martin *et al.*, *Nat. Genet.* **39**, 733 (2007).
7. J. Fellay *et al.*, *PLoS Genet.* **5**, e1000791 (2009).
8. J. Fellay *et al.*, *Science* **317**, 944 (2007).
9. W. M. Brown *et al.*, *Diabetes Obes. Metab.* **11** (suppl. 1), 2 (2009).
10. S. Leslie, P. Donnelly, G. McVean, *Am. J. Hum. Genet.* **82**, 48 (2008).
11. K. J. Smith *et al.*, *Immunity* **4**, 215 (1996).
12. A. Kosmrlj *et al.*, *Nature* **465**, 350 (2010).
13. W. Fischer *et al.*, *PLoS ONE* **5**, e12303 (2010).
14. E. L. Turnbull *et al.*, *J. Immunol.* **176**, 6130 (2006).
15. J. Martinez-Picado *et al.*, *J. Virol.* **80**, 3617 (2006).

10.1126/science.1200035

GEOCHEMISTRY

Earth's Second Wind

Lee R. Kump

One of the great mysteries of Earth history is why, after 3 billion years of evolution, about 600 million years ago (Ma), organisms emerged from the microscopic world and shortly thereafter developed skeletons and shells (1). Two prevalent hypotheses include climate cooling (2) and an increase in the oxygen content of the ocean (3). To date, the best evidence in support of the oxygen theory has been the size of animals themselves: Larger animals have higher oxygen demands that can only be met by oxygen-rich waters. Now Dahl *et al.* (4) present independent proxy evidence for an increase of oceanic (and by inference, atmospheric) oxygen concentrations—from levels below the lower limit for large animals, to levels above—at about 550 Ma. This second rise of oxygen follows nearly 2 billion years after the well-documented first “Great Oxidation Event” (GOE) at about 2400 Ma.

The proxy used by these researchers is the isotopic composition of marine aqueous molybdenum (Mo), preserved in sedimentary rocks rich in organic matter and pyrite (an iron sulfide mineral) (see the figure, panel A). The ocean receives Mo from the weathering of rocks on land and deposits it in sediments. Under reducing conditions such as existed in soil environments before 2400 Ma, Mo is immobile.

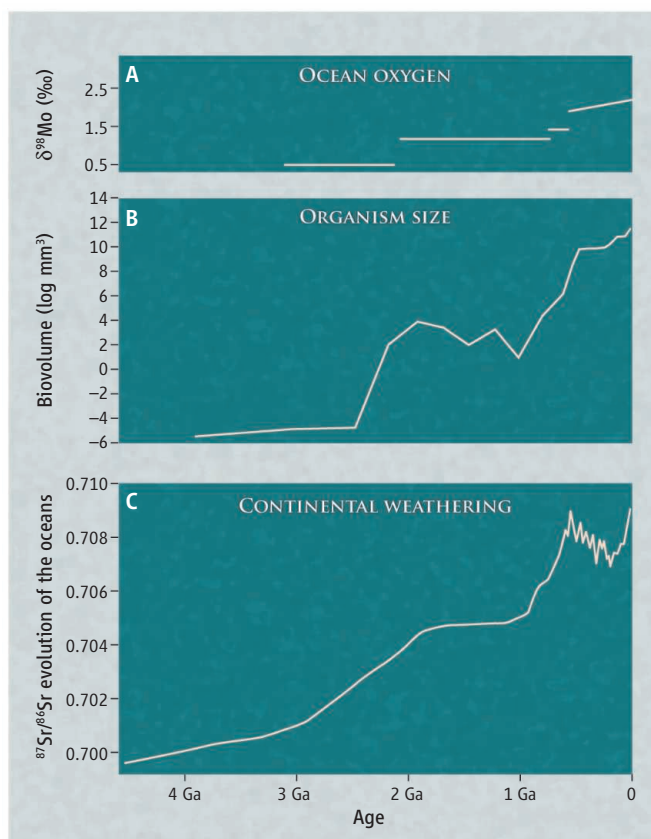
In the presence of oxygen, however, it forms the soluble molybdate ion and can be mobilized from soils into river waters and delivered to the ocean.

Although Mo accumulates in an oxygen-

Studies of molybdenum isotopes in sediments support the idea that increases in atmospheric oxygen in the Devonian period drove increases in animal size.

ated ocean, it ultimately is removed into sediments. These sedimentary sinks exist in well-oxygenated conditions, where Mo is removed with Fe and Mn oxides (the oxic sink), and in basins where reducing conditions prevail, and Mo is converted to thiomolybdate species that are scavenged into organic matter and sulfide minerals (the reducing sink). The oxic sink accumulates more of the lighter isotopes of Mo, whereas the reducing sink quantitatively removes the isotopes in their ambient proportions. When oxic sinks are scarce, the Mo isotopic composition of the ocean (and in reducing sediments) reflects the riverine input from recent continental weathering, whereas when oxic sinks are abundant (as they are today), the ocean's isotope ratio of heavy (^{98}Mo) to light (^{95}Mo) increases. Samples from the (relatively scarce) reducing sediments faithfully record the oxic ocean's “heavy” isotopic composition.

A compilation of existing analyses of Mo isotopic compositions of rocks of all ages (4) indicates low ratios until 2.7 billion years ago (Ga)—that is, prior to the GOE—when the ratio of $^{98}\text{Mo}/^{95}\text{Mo}$ increased from 0.7 (equivalent to the presumed riverine input) to 1.1 (5). This value was sustained through much of the Proterozoic (4) except for a dip between 2.4 and 2.2 Ga tied perhaps to glaciation (6). The isotope ratio shifted more posi-



Oxygen in the oceans and the atmosphere. Several proxies suggest an increase in atmospheric oxygen about 550 Ma. (A) An increase in the standardized $^{98}\text{Mo}/^{95}\text{Mo}$ ratio of the oceans through time reflects the increased oxygenation of the ocean (4). (B) The increase in the size of organisms recorded in the fossil record is shown (1). (C) The evolution of the Sr isotopic composition of the ocean (6) shows a stepwise increase in the $^{87}\text{Sr}/^{86}\text{Sr}$, which reflects the increasing influence of continental weathering over time on the composition of the ocean.

Department of Geosciences, Pennsylvania State University, University Park, PA 16802, USA. E-mail: lkump@psu.edu

tive in the Neoproterozoic (~550 Ma) to a value of about 1.4. These values persisted until the Devonian, when the ratio increased to about 2.0. Dahl *et al.* attribute the increase to the development of terrestrial plant ecosystems and link it to an increase in the size of fossil predatory fish. The proportion of inertinite (mostly in the form of charcoal) in coals increased markedly at this time. This supports an increase in atmospheric oxygen in the sense that higher oxygen levels promote more widespread and frequent wildfires (7). The record is sparse between the Devonian and today, but there seems to be a progressive increase to the modern value of 2.3 that could reflect continued increases in ocean oxygen levels to the present.

The stepwise oxygenation of the ocean (and perhaps the atmosphere as well) interpreted from the Mo isotope data bears a striking resemblance both to the maximum size of organisms through time, as expected (see the figure, panel B), and also to the isotopic composition of strontium (Sr) in the ocean (see the figure, panel C). The Sr isotope curve is best interpreted in terms of an increasing proportion of Sr derived from continental weathering that entered the ocean. Along with Sr came phosphorus (P), the weathering-derived bioessential element that is generally thought

to limit the burial rate of organic matter and thus the rate of oxygen production (8).

The carbon isotopic composition ($\delta^{13}\text{C}$) of the ocean, as reflected in the carbonates and organic matter deposited from it, provides a proxy for the proportional burial of organic matter. This record shows a general trend through time that would support progressively higher burial rates of organic matter and oxygen production (9). The interval of increasing body size, oxygenation, and continental weathering (Neoproterozoic, 1000 to 542 Ma) is indeed characterized by high values of $\delta^{13}\text{C}$ (punctuated by sharp drops, which was the subject of an entire session at this fall's Annual Meeting of the Geological Society of America) (10). The higher P input and oxygen production rates indicated by the Sr and C isotopes would have driven oxygen levels up, but negative feedback would have quickly established a higher baseline oxygen level in the ocean that brought the C, P, and O_2 cycles back into balance through more efficient organic matter recycling in the ocean during early diagenesis and during weathering.

A broadly self-consistent picture of a stepwise oxygenation of the oceans seems to be emerging, but the link to the atmospheric evolution remains less clear. As the oceanic

anoxic intervals during the past 150 million years indicate, the atmospheric and oceanic oxygen status at any particular time can be quite distinct, as can their evolutionary histories (11). Only through a broadly integrated analysis of both atmospheric and oceanic proxies, using models that explicitly consider the links between atmosphere and ocean oxygenation, will we fully understand the coevolution of oceanic and atmospheric oxygenation through Earth history.

References

1. J. L. Payne *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 24 (2009).
2. D. Schwartzman, *Life, Temperature, and the Earth* (Columbia Univ. Press, New York, 1999).
3. B. Runnegar, *Alcheringa* **6**, 223 (1982).
4. T. W. Dahl *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 17911 (2010).
5. C. Siebert, J. D. Kramers, Th. Meisel, Ph. Morel, Th. F. Nägler, *Geochim. Cosmochim. Acta* **69**, 1787 (2005).
6. M. Wille *et al.*, *Geochim. Cosmochim. Acta* **71**, 2417 (2007).
7. I. J. Glasspool, A. C. Scott, *Nat. Geosci.* **3**, 627 (2010).
8. G. A. Shields, *eEarth Discuss.* **2**, 69 (2007).
9. J. M. Hayes, J. R. Waldbauer, *Philos. Trans. R. Soc. B* **361**, 931 (2006).
10. L. A. Derry, T. Lyons, convenors, Understanding high-amplitude stable isotope variations in Proterozoic strata, Annual Meeting of the Geological Society of America, Denver, 31 October to 3 November, Session T79 (2010).
11. N. J. Butterfield, *Geobiology* **7**, 1 (2009).

10.1126/science.1199919

CELL BIOLOGY

Gett'N-WASP Stripes

Mathias Gautel and Elisabeth Ehler

Sarcomeres, the smallest contractile units of striated muscle cells, have regular structures, but are constantly renewing their filaments. It has been difficult, however, to explain how the tens of thousands of sarcomeres present in many muscle cells carry out the apparently incompatible tasks of performing contractile work while rebuilding their machinery. On page 1536 of this issue, Takano *et al.* (1) identify a new player in the process: N-WASP, a protein involved in regulating the assembly of muscle actin filaments. The finding could help researchers better understand muscle cell enlargement (hypertrophy) and muscle-related disease.

Sarcomere formation requires the precise alignment and coordinated integration

of actin and myosin filaments (2). This is achieved by the action of at least two giant scaffold proteins, titin (also known as connectin) and nebulin. Titin integrates myosin filaments, provides an elastic link in the sarcomere, and has signaling functions related to the turnover of myofibrils in response to mechanical load (3, 4). In contrast, nebulin is firmly associated with actin filaments due to direct interaction via its more than 180 small helical repeats (5). In addition, nebulin reportedly interacts with two proteins, tropomodulin and CapZ, that are involved in capping the pointed and barbed ends of growing actin filaments (4). However, the extremely regular sarcomere structure is not static. Myofibrillar proteins turn over continually, as the composition of myofibrils changes, and damaged proteins are exchanged (6).

Ordered actin assembly requires the nucleation, elongation, and capping of actin filaments (7). In muscle, this requires additional

The N-WASP protein is involved in regulating the assembly of muscle actin filaments.

coordination with the assembly of structures known as Z-disks; the complex must then incorporate titin, and finally myosin filaments. Although the barbed ends of actin filaments serve as the major site for rapid growth, this growth has to be tightly regulated in sarcomeres to maintain a para-crystalline order, which is a prerequisite for its effective function as a linear motor. The CapZ protein seems to be the major actin-capping factor at the Z-disk. However, the nebulin interactions that direct CapZ to the Z-disk are controversial (8, 9).

Now, Takano *et al.* show that N-WASP—a ubiquitously expressed member of the Wiscott-Aldrich-Syndrome Protein (WASP) family—is involved in actin assembly in muscle. Past studies have shown that WASP proteins are involved in cell migration, and are nucleation-promoting factors that cooperate with other factors like the Arp2/3 protein complex to initiate actin filament assembly (7). In their

King's College London British Heart Foundation Centre of Research Excellence, Cardiovascular Division and Randall Division for Cell and Molecular Biophysics, London, SE1 1UL, UK. E-mail: mathias.gautel@kcl.ac.uk

tive in the Neoproterozoic (~550 Ma) to a value of about 1.4. These values persisted until the Devonian, when the ratio increased to about 2.0. Dahl *et al.* attribute the increase to the development of terrestrial plant ecosystems and link it to an increase in the size of fossil predatory fish. The proportion of inertinite (mostly in the form of charcoal) in coals increased markedly at this time. This supports an increase in atmospheric oxygen in the sense that higher oxygen levels promote more widespread and frequent wildfires (7). The record is sparse between the Devonian and today, but there seems to be a progressive increase to the modern value of 2.3 that could reflect continued increases in ocean oxygen levels to the present.

The stepwise oxygenation of the ocean (and perhaps the atmosphere as well) interpreted from the Mo isotope data bears a striking resemblance both to the maximum size of organisms through time, as expected (see the figure, panel B), and also to the isotopic composition of strontium (Sr) in the ocean (see the figure, panel C). The Sr isotope curve is best interpreted in terms of an increasing proportion of Sr derived from continental weathering that entered the ocean. Along with Sr came phosphorus (P), the weathering-derived bioessential element that is generally thought

to limit the burial rate of organic matter and thus the rate of oxygen production (8).

The carbon isotopic composition ($\delta^{13}\text{C}$) of the ocean, as reflected in the carbonates and organic matter deposited from it, provides a proxy for the proportional burial of organic matter. This record shows a general trend through time that would support progressively higher burial rates of organic matter and oxygen production (9). The interval of increasing body size, oxygenation, and continental weathering (Neoproterozoic, 1000 to 542 Ma) is indeed characterized by high values of $\delta^{13}\text{C}$ (punctuated by sharp drops, which was the subject of an entire session at this fall's Annual Meeting of the Geological Society of America) (10). The higher P input and oxygen production rates indicated by the Sr and C isotopes would have driven oxygen levels up, but negative feedback would have quickly established a higher baseline oxygen level in the ocean that brought the C, P, and O_2 cycles back into balance through more efficient organic matter recycling in the ocean during early diagenesis and during weathering.

A broadly self-consistent picture of a stepwise oxygenation of the oceans seems to be emerging, but the link to the atmospheric evolution remains less clear. As the oceanic

anoxic intervals during the past 150 million years indicate, the atmospheric and oceanic oxygen status at any particular time can be quite distinct, as can their evolutionary histories (11). Only through a broadly integrated analysis of both atmospheric and oceanic proxies, using models that explicitly consider the links between atmosphere and ocean oxygenation, will we fully understand the coevolution of oceanic and atmospheric oxygenation through Earth history.

References

1. J. L. Payne *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 24 (2009).
2. D. Schwartzman, *Life, Temperature, and the Earth* (Columbia Univ. Press, New York, 1999).
3. B. Runnegar, *Alcheringa* **6**, 223 (1982).
4. T. W. Dahl *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 17911 (2010).
5. C. Siebert, J. D. Kramers, Th. Meisel, Ph. Morel, Th. F. Nägler, *Geochim. Cosmochim. Acta* **69**, 1787 (2005).
6. M. Wille *et al.*, *Geochim. Cosmochim. Acta* **71**, 2417 (2007).
7. I. J. Glasspool, A. C. Scott, *Nat. Geosci.* **3**, 627 (2010).
8. G. A. Shields, *eEarth Discuss.* **2**, 69 (2007).
9. J. M. Hayes, J. R. Waldbauer, *Philos. Trans. R. Soc. B* **361**, 931 (2006).
10. L. A. Derry, T. Lyons, convenors, Understanding high-amplitude stable isotope variations in Proterozoic strata, Annual Meeting of the Geological Society of America, Denver, 31 October to 3 November, Session T79 (2010).
11. N. J. Butterfield, *Geobiology* **7**, 1 (2009).

10.1126/science.1199919

CELL BIOLOGY

Gett'N-WASP Stripes

Mathias Gautel and Elisabeth Ehler

Sarcomeres, the smallest contractile units of striated muscle cells, have regular structures, but are constantly renewing their filaments. It has been difficult, however, to explain how the tens of thousands of sarcomeres present in many muscle cells carry out the apparently incompatible tasks of performing contractile work while rebuilding their machinery. On page 1536 of this issue, Takano *et al.* (1) identify a new player in the process: N-WASP, a protein involved in regulating the assembly of muscle actin filaments. The finding could help researchers better understand muscle cell enlargement (hypertrophy) and muscle-related disease.

Sarcomere formation requires the precise alignment and coordinated integration

of actin and myosin filaments (2). This is achieved by the action of at least two giant scaffold proteins, titin (also known as connectin) and nebulin. Titin integrates myosin filaments, provides an elastic link in the sarcomere, and has signaling functions related to the turnover of myofibrils in response to mechanical load (3, 4). In contrast, nebulin is firmly associated with actin filaments due to direct interaction via its more than 180 small helical repeats (5). In addition, nebulin reportedly interacts with two proteins, tropomodulin and CapZ, that are involved in capping the pointed and barbed ends of growing actin filaments (4). However, the extremely regular sarcomere structure is not static. Myofibrillar proteins turn over continually, as the composition of myofibrils changes, and damaged proteins are exchanged (6).

Ordered actin assembly requires the nucleation, elongation, and capping of actin filaments (7). In muscle, this requires additional

The N-WASP protein is involved in regulating the assembly of muscle actin filaments.

coordination with the assembly of structures known as Z-disks; the complex must then incorporate titin, and finally myosin filaments. Although the barbed ends of actin filaments serve as the major site for rapid growth, this growth has to be tightly regulated in sarcomeres to maintain a para-crystalline order, which is a prerequisite for its effective function as a linear motor. The CapZ protein seems to be the major actin-capping factor at the Z-disk. However, the nebulin interactions that direct CapZ to the Z-disk are controversial (8, 9).

Now, Takano *et al.* show that N-WASP—a ubiquitously expressed member of the Wiscott-Aldrich-Syndrome Protein (WASP) family—is involved in actin assembly in muscle. Past studies have shown that WASP proteins are involved in cell migration, and are nucleation-promoting factors that cooperate with other factors like the Arp2/3 protein complex to initiate actin filament assembly (7). In their

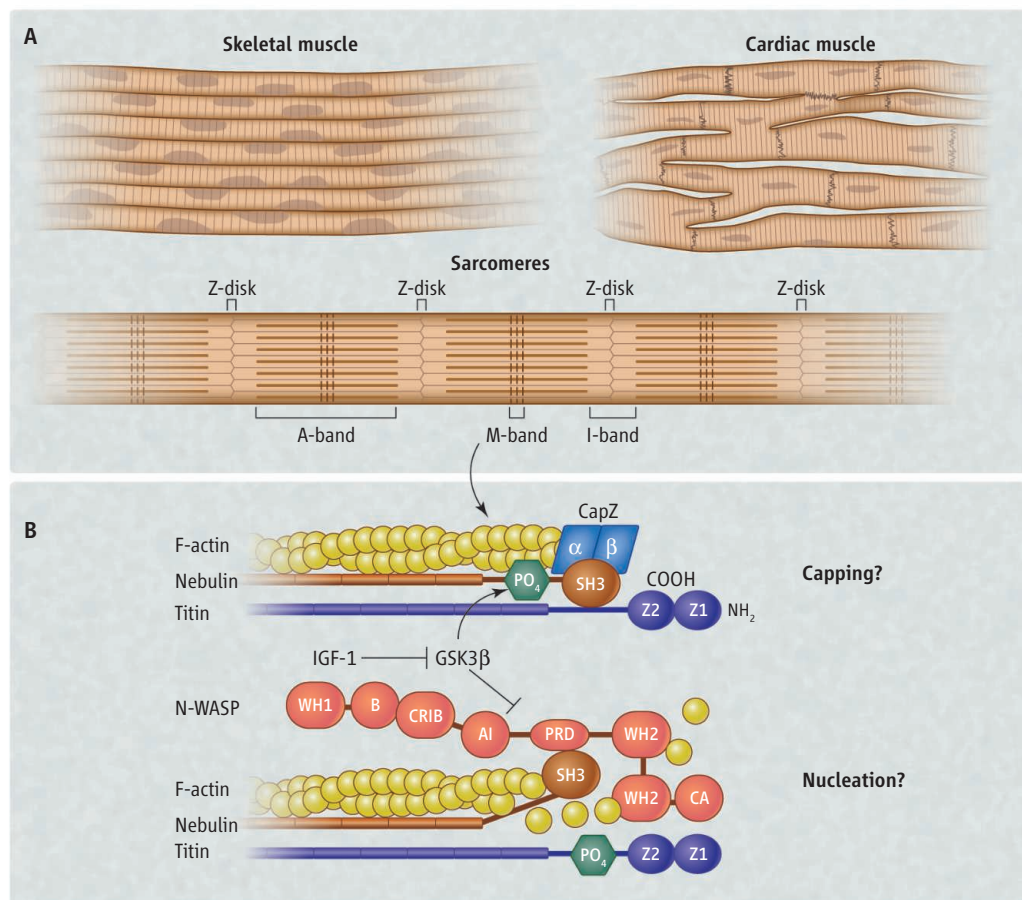
King's College London British Heart Foundation Centre of Research Excellence, Cardiovascular Division and Randall Division for Cell and Molecular Biophysics, London, SE1 1UL, UK. E-mail: mathias.gautel@kcl.ac.uk

study, Takano *et al.* show that the proline-rich regions of N-WASP interact with nebulin's C-terminal Src homology 3 (SH3) domain, and that N-WASP is rapidly recruited to the Z-disk after treatment with insulin-like growth factor 1 (IGF-1), which stimulates myofibrillogenesis and suppresses sarcomere breakdown via the PI3K-Akt pathway (6). N-WASP then co-localizes with fluorescently labeled actin as it is incorporated toward the pointed ends of actin filaments. Knocking down N-WASP expression in cultured myofibers prevents IGF-1-induced incorporation of the labeled actin into cell structures known as I-bands. Long-term knockdown of N-WASP in mice leads to pronounced and IGF-1 insensitive decrease of muscle fiber size, suggesting that this pathway is also relevant for the *in vivo* regulation of muscle maintenance and hypertrophy.

These results suggest that N-WASP is an important regulator of actin assembly in muscle. Supporting this view, knockout mice lacking the N-WASP gene die before embryonic day 11, with obvious signs of heart failure (non-contractile, dilated hearts; however, some contracting cardiomyocytes could apparently be isolated from embryos) (10). Similarly, nebulin knockout mice, although initially viable, develop symptoms that also indicate defects in actin capping; e.g., they contain broad Z-disk aggregates or thread-like structures (known as nemaline rods) that have been associated with a congenital neuromuscular disorder. These mice also show severe growth retardation, myopathy, and muscle weakness (9, 11), suggesting that secondary myofibril assembly might be impaired.

A number of questions should now be addressed, related to accessory factors, factors required for elongation, and even the function of the multiple actin binding domains [WH-2 domains (7)] found in WASP. What is the interplay with other actin regulators, such as leiomodin, which were initially assumed to be crucial for actin filament maintenance (12) and may be part of the proposed activity-driven length regulation of actin filaments (13)?

The role of N-WASP in development also



Muscle assembly. (A) A sarcomere is defined as the region between the two Z-disks, where actin filaments are inserted. Myosin filaments are anchored at the M-band. (B) Hypothetical model of actin filament capping (above). In the assembled state, interaction between the SH3 domain of phosphorylated nebulin with titin and CapZ prevents the addition of actin monomers (yellow circles). In actin nucleation (below), phosphorylation of titin and release of nebulin's SH3 domain, when glycogen-synthase kinase (GSK3 β)-driven phosphorylation is suppressed, may allow interaction between nebulin and N-WASP and facilitate nucleation and synthesis of new filaments. Abbreviations for N-WASP domains as in Takano *et al.*

remains unclear. The heart and muscle phenotype of the N-WASP knockout animals reported by Snapper *et al.* (10) were not characterized very deeply, so it is not known how important N-WASP is for initial myofibril assembly. It is astonishing, that the IGF1-induced N-WASP recruitment appeared to occur only in preexisting sarcomeres, apparently without the assembly of primary new myofibrils in parallel. Is N-WASP thus inducing radial growth from the perimeter of the myofibril? Higher image resolution in living cells may help answer these questions. Finally, the phosphorylation-dependent regulation of capping and nucleation events at the Z-disk by IGF-1 regulated kinase pathways remain to be further elucidated. How does phosphorylation of the N-terminal segment preceding nebulin's SH3 domain regulate ligand binding? Is the affinity of nebulin "switched" between CapZ in assembled sarcomeres, and N-WASP during myofibril assembly (see the figure)? What role does

the titin N terminus (9) play in this process?

Having painted the sarcomere with broad WASP-stripes, the dynamic patterns during hypertrophy and atrophy will now need to be sketched in more detail.

References

1. K. Takano *et al.*, *Science* **330**, 1536 (2010).
2. J. W. Sanger *et al.*, *J. Muscle Res. Cell Motil.* **26**, 343 (2005).
3. S. Lange, E. Ehler, M. Gautel, *Trends Cell Biol.* **16**, 11 (2006).
4. A. Kontogianni-Konstantopoulos, M. A. Ackermann, A. L. Bowman, S. V. Yap, R. J. Bloch, *Physiol. Rev.* **89**, 1217 (2009).
5. M. Pfuhl, S. J. Winder, A. Pastore, *EMBO J.* **13**, 1782 (1994).
6. M. Sandri, *Physiology (Bethesda)* **23**, 160 (2008).
7. K. G. Campellone, M. D. Welch, *Nat. Rev. Mol. Cell Biol.* **11**, 237 (2010).
8. C. T. Pappas, N. Bhattacharya, J. A. Cooper, C. C. Gregorio, *Mol. Biol. Cell* **19**, 1837 (2008).
9. C. C. Witt *et al.*, *EMBO J.* **25**, 3843 (2006).
10. S. B. Snapper *et al.*, *Nat. Cell Biol.* **3**, 897 (2001).
11. M. L. Bang *et al.*, *J. Cell Biol.* **173**, 905 (2006).
12. D. Chereau *et al.*, *Science* **320**, 239 (2008).
13. R. Littlefield, V. M. Fowler, *Annu. Rev. Cell Dev. Biol.* **14**, 487 (1998).

10.1126/science.1199920

CREDIT: ADAPTED BY P. HUEY/SCIENCE

RETROSPECTIVE

Veronica Rodrigues (1953–2010)

K. VijayRaghavan¹ and Michael Bate²

With the death of Veronica Rodrigues on 10 November, India has lost a leading figure in the resurgence of research and teaching in the biological sciences in the subcontinent. Her scientific influence was widely felt, but she was also intimately connected with fostering and advancing research in molecular biology and developmental genetics at the Tata Institute of Fundamental Research (TIFR) in Mumbai, and more recently in the inception of the landmark center of excellence at the National Centre for Biological Sciences (NCBS) in Bangalore. Her leadership was particularly apparent in Indian developmental biology and was especially inspirational to women scientists.

Veronica Rodrigues was born in 1953 in Nairobi, one of 10 children of Goan immigrants of very modest means. She entered Makerere University in Kampala but then, in the turmoil surrounding the regime of Idi Amin, moved to Trinity College, Dublin, where she earned an undergraduate degree in microbiology in 1976. Stimulated by the pioneering studies of the neurogeneticist Obaid Siddiqi on bacterial genetics, Veronica applied to work with him at the Tata Institute in Mumbai. In 1976, she landed in India (stateless, but carrying a singular British passport of the time that barred entry into Britain), a country about which she later confessed she had a somewhat naïve view as a cultural paradise. By this time, Siddiqi had shifted his interests to neurogenetics, and Veronica was recruited to investigate olfaction in the fruit fly *Drosophila melanogaster*. So important was her contribution to the study of olfactory mutants that while she was a Ph.D. student, she was offered a permanent faculty position at the TIFR. She spent 3 years on leave from the institute (1982 to 1984) to work at the Max Planck Institute for Biological Cybernetics in Tübingen, where she performed a landmark study of odor coding in the antennal lobe of the fly.

On returning to the TIFR, Veronica combined her experience in neurobiology and behavior with her expertise in genetics and development and forged an influential group of researchers and students to study the for-



mation of olfactory circuitry. She and her collaborators pioneered research into chemosensory biology. Her standard behavioral and electrophysiological assays for adult and larval responses to olfactory and gustatory cues are still widely used in the field, and variants of these assays have seen a major revival with the advent of new genetic and molecular tools for studying the neuronal circuitry underlying patterns of behavior. Her work and expertise ranged from genes and molecules to behavior, showing how each step in the assembly of the olfactory neuronal network is related to the emergence and maintenance of function. In daring to take on such ambitious projects, Veronica often started with students who were completely inexperienced in experimental biology. Under her tutelage, these raw recruits blossomed into self-confident mature researchers, setting out to become part of a worldwide network of scientists who began their careers in the Rodrigues lab.

Perhaps her greatest achievement was fostering young scientific talent not only in Mumbai, but more widely through her leadership in creating and running an influential biennial course in neurobiology at the International Centre for Theoretical Physics in Trieste. Through this course, which she led for the past 9 years, she inspired a new generation of students from countries as far apart as Iran and Cuba and more especially from Africa. In parallel with her research and teaching, Veronica gently assumed the leadership of

A charismatic developmental biologist inspired a new generation of scientists and research institutes in India.

the Molecular Biology Unit in the TIFR, later to become the Department of Biological Sciences. Over 25 years, the department grew in its research scope and capacity, nurturing high-quality scientists who can now be found in laboratories worldwide. It also spawned, in 1988, the NCBS, as a separate TIFR center. In 1992, the NCBS moved to a new campus in Bangalore. There, Veronica continued to act as a responsible steward, navigating the transition through tense situations that might otherwise have prevented the formation of strong bonds between geographically distinct parts of the TIFR. Despite this commitment to launching the NCBS in Bangalore, she chose to remain in her beloved TIFR campus in Mumbai. However, the diagnosis of her cancer led to an eventual move to Bangalore where the management of her health was easier.

Veronica's direct influence on the entire academic community in Bangalore and Mumbai was enormous. Although she was a tough scientific critic and always spoke openly and honestly (she held nothing back), Veronica inspired loyalty and affection because she was usually right. She was keenly aware of India's male-dominated scientific environment, yet still achieved great success as a scientist, mentor (particularly of women scientists), colleague, and leader. She was proud of other contributions to Indian science on all these fronts.

To many Western scientists, Veronica Rodrigues was their gateway to India, her care and her generosity smoothing the path for their first stumbling steps in very unfamiliar surroundings. To know Veronica was to enter a world of very particular places and occasions, where the pursuit of science was indissolubly linked to the enjoyment of good conversation, food, and drink. A tea stall on Colaba Causeway, a bar in the shadow of the Taj Hotel, an ice cream parlor in Trieste were the inescapable backdrops for discussion, the development of new ideas, and the simple celebration of life.

Stylish without effort and full of impish fun, her premature departure leaves her wide network of friends bereft. Her imprint on Indian science will endure, and the story of the stateless woman from Africa whom chance brought to Mumbai will be an inspiration to all who follow her.

10.1126/science.1200787

CREDIT: APURVA SARIN

¹National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bangalore, India. ²Department of Zoology, Cambridge University, Cambridge CB2 3EJ, UK. E-mail: vijay@ncbs.res.in

Scenarios for Global Biodiversity in the 21st Century

Henrique M. Pereira,^{1*†} Paul W. Leadley,^{2*} Vânia Proença,¹ Rob Alkemade,³ Jörn P. W. Scharlemann,⁴ Juan F. Fernandez-Manjarrés,² Miguel B. Araújo,^{5,6} Patricia Balvanera,⁷ Reinette Biggs,⁸ William W. L. Cheung,⁹ Louise Chini,¹⁰ H. David Cooper,¹¹ Eric L. Gilman,¹² Sylvie Guénette,¹³ George C. Hurtt,^{10,14} Henry P. Huntington,¹⁵ Georgina M. Mace,¹⁶ Thierry Oberdorff,¹⁷ Carmen Revenga,¹⁸ Patrícia Rodrigues,¹ Robert J. Scholes,¹⁹ Ussif Rashid Sumaila,¹³ Matt Walpole⁴

Quantitative scenarios are coming of age as a tool for evaluating the impact of future socioeconomic development pathways on biodiversity and ecosystem services. We analyze global terrestrial, freshwater, and marine biodiversity scenarios using a range of measures including extinctions, changes in species abundance, habitat loss, and distribution shifts, as well as comparing model projections to observations. Scenarios consistently indicate that biodiversity will continue to decline over the 21st century. However, the range of projected changes is much broader than most studies suggest, partly because there are major opportunities to intervene through better policies, but also because of large uncertainties in projections.

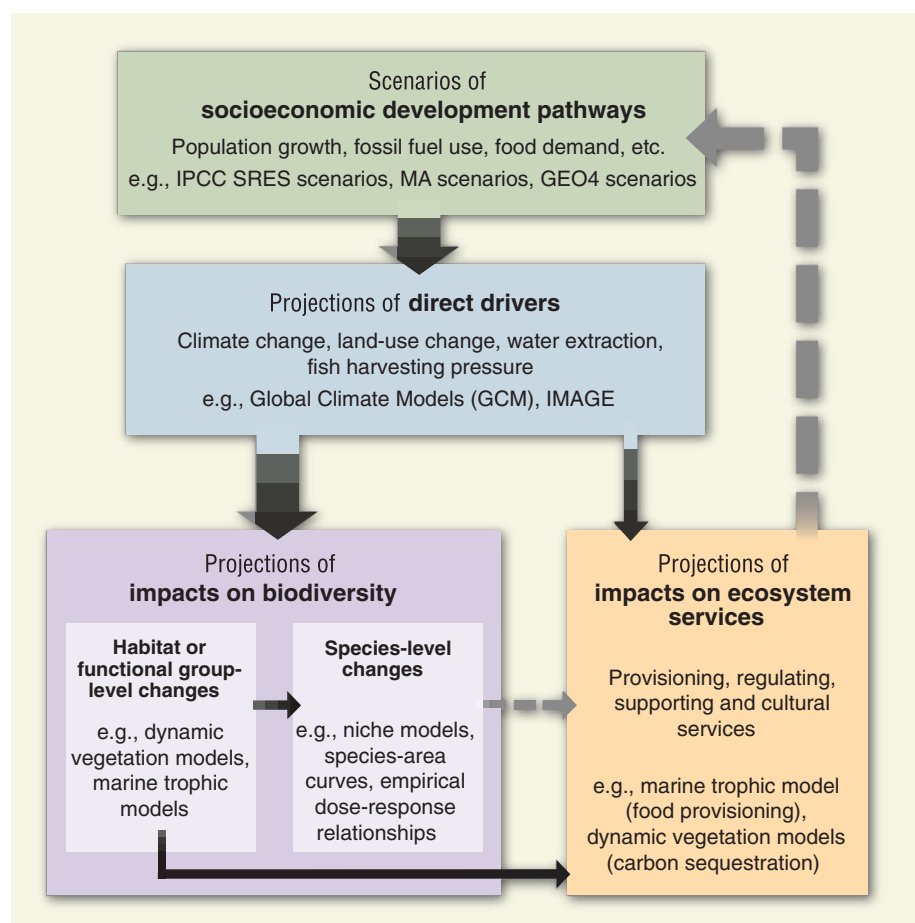


Fig. 1. Overview of methods and models commonly used for constructing biodiversity scenarios. Some models include several components of this figure, such as the integrated assessment model IMAGE (1) or the marine trophic model “Ecosim with Ecopath” (23). Black arrows indicate key linkages treated in biodiversity scenarios. Dashed gray arrows indicate linkages that are absent in current biodiversity scenarios. In some cases, impacts on ecosystem services may be mediated by changes in the abiotic condition of ecosystems (thin arrow from direct drivers to ecosystem services).

Quantitative estimates of the future trajectories of biodiversity, which we broadly refer to as biodiversity scenarios, are typically based on the coupling of several complex components (Fig. 1). Socioeconomic scenarios with trajectories of key indirect drivers of ecological change, such as human population growth and greenhouse gas emissions, are developed under different assumptions regarding society’s development, often associated with “storylines” (1). These trajectories are then fed into models that project changes in direct drivers of ecosystem change, such as climate and land-use change, in different regions of the world (1, 2). Finally, the projected drivers are used as inputs to biodiversity models (Table 1). In some cases, associated changes in key ecosystem services are also modeled, although quantifying the link between biodiversity and ecosystem services remains a major scientific challenge (3, 4). Here, we review recent model-based biodiversity scenarios, which have grown rapidly in number over the last few years owing to major advances in modeling and data availability.

Biodiversity change has many metrics (5). Here we group these metrics into four classes: species extinctions, species abundance and community structure, habitat loss and degradation, and shifts in the distribution of species and biomes. Scenarios of species extinction risk (6, 7) address

¹Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal. ²Laboratoire Écologie, Systématique et Évolution, UMR 8079 CNRS, Université Paris-Sud 11, F-91405 Orsay, France. ³Netherlands Environmental Assessment Agency (PBL), Post Office Box 303, 3720 AH Bilthoven, Netherlands. ⁴United Nations Environment Programme World Conservation Monitoring Centre, 219 Huntingdon Road, Cambridge CB3 0DL, UK. ⁵Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Científicas, calle Jose Gutierrez Abascal, 28006 Madrid, Spain. ⁶Cátedra Rui Nabeiro-Biodiversidade, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade de Évora, Largo dos Colegiais, 7000 Évora, Portugal. ⁷Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, Apartado Postal 27-3, Sta. Maria de Guido, C.P. 58090, Morelia, Michoacán, México. ⁸Stockholm Resilience Center, Stockholm University, Stockholm, Sweden 10691. ⁹School of Environmental Sciences, University of East Anglia, Norwich NR4 7TJ, UK. ¹⁰Department of Geography, University of Maryland, College Park, MD 20742, USA. ¹¹Secretariat of the Convention on Biological Diversity, World Trade Center, 413 St. Jacques Street, Suite 800, Montreal, Quebec, Canada H2Y 1N9. ¹²College of Natural and Computational Sciences, Hawaii Pacific University, Honolulu, HI 96822, USA. ¹³Fisheries Centre, Aquatic Ecosystems Research Laboratory (AERL), 2202 Main Mall, The University of British Columbia, Vancouver, BC, Canada V6T 1Z4. ¹⁴Pacific Northwest National Laboratory, Joint Global Change Research Institute, College Park, MD 20740, USA. ¹⁵The Pew Environment Group, 23834 The Clearing Drive, Eagle River, AK 99577, USA. ¹⁶Centre for Population Biology, Imperial College London, Silwood Park, Ascot, SL5 7PY, UK. ¹⁷Laboratoire de Biologie des Organismes et Écosystèmes Aquatiques, UMR IRD 207 Muséum National d’Histoire Naturelle, 43 rue Cuvier, 75005 Paris, France. ¹⁸The Nature Conservancy, 4245 North Fairfax Drive, Arlington, VA 22203, USA. ¹⁹CSIR Natural Resources and Environment, Post Office Box 395, Pretoria 0001, South Africa.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: hpereira@fc.ul.pt

the irreversible component of biodiversity change, but species extinctions have weak links to ecosystem services and respond less rapidly to global change than do other metrics (e.g., the range of a species can decline shortly after habitat change, but that species may not become extinct as a result) (8). More-responsive metrics include changes in species abundances and community structure and, at a higher organizational level, habitat loss or biome changes. At both the species and ecosystem levels, many of the projected changes can best be described as shifts in potential distribution, with their current favorable conditions vanishing in some places, which may cause local extinctions, and appearing in new places, which may result in colonizations.

Models used to estimate global change impacts on biodiversity vary substantially in complexity and underlying hypotheses (Fig. 1 and Table 1), but can be broadly classified into phenomenological or process-based models. Phenomenological models rely on empirical relationships between environmental variables and a biodiversity metric (9). One of the simplest phenomenological models is to subtract future land-cover changes from a species' current distribution to estimate extinction risk (6). Species-area models use the empirical relationship between area and species

number to estimate species committed to extinction after habitat loss (10). Niche-based models (or bioclimatic envelope models) use statistical relationships between current species distributions and environmental variables, such as temperature and precipitation, to project the future distribution of a species under climate change (11). Dose-response relationships depend on experimental or observational data to estimate the impacts of drivers on biodiversity, e.g., the effect of nitrogen deposition on mean species abundance (12, 13). Process-based models simulate processes such as population growth or mechanisms such as ecophysiological responses (14). Dynamic global vegetation models (DGVMs), which play an important role in many scenarios, are complex ecosystem models integrating processes such as photosynthesis, respiration, plant competition for resources, and biogeochemical cycles (15). Marine trophic models simulate the biomass dynamics at different levels of the trophic web using mass-balance equations and can be used to assess the population impacts of harvesting (16).

Here we review global-scale biodiversity scenarios for each of the four biodiversity metrics outlined above. We analyze sources of variation within and between scenarios, coherence between

models and observations, links between biodiversity and ecosystem services, and relevance of scenarios to policy.

Species Extinctions

Scenarios for terrestrial ecosystems project that future species extinction rates will greatly surpass background rates estimated from the cenozoic fossil record (17) and could exceed recent rates of extinction by more than two orders of magnitude (Fig. 2 and table S1). There is great variation in projected future extinction rates both within and between studies, with three factors explaining much of this variation. First, the degree of land-use and climate change explains a substantial fraction of the range of projected extinctions within studies [e.g., projected vertebrate extinctions are 11 to 34% for 0.8° to 1.7°C global warming versus 33 to 58% for >2.0°C warming in (7)], indicating that limitation of land-use change, especially in tropical and subtropical regions, and aggressive climate mitigation could substantially reduce extinction risks. Second, an important contribution to the broad range of projections within studies is a lack of understanding of species ecology, especially migration rates [e.g., the highest projected extinction rates are 38% with unlimited migrations rates versus 58% with no migration in (7)]

Table 1. Examples of biodiversity scenario studies highlighting methods used to calculate impacts of global change on several biodiversity metrics. Socioeconomic scenarios: Millennium Ecosystem Assessment (MA), Global Biodiversity Outlook 2 (GBO2), Global Environmental Outlook 4 (GEO4), IPCC Special Report on Emission Scenarios (IPCC SRES), International Assessment

for Agricultural Science, Technology and Development (IAASTD). Direct drivers: land-use change (LUC), climate change (CC), nitrogen deposition (N), water use, and fishing effort. Projections of direct drivers: indicates model that was used to simulate future changes in direct drivers (GCM, General Circulation Model, with specific climate model indicated in parentheses).

Study	Socioeconomic scenarios	Direct drivers	Projections of direct drivers	Projections of impacts on biodiversity	Metrics of biodiversity and ecosystem services	Year
<i>Terrestrial</i>						
(38)	MA	LUC, CC	IMAGE	Species-area relationships	Species extinctions (plants) and habitat loss	2100
(7)	IPCC SRES and others	CC	GCM (HadCM2)	Niche-based models. Range changes converted to extinction risk using species-area curves or IUCN status	Species extinctions (plants and animals)	2050
(6)	MA	LUC, CC	IMAGE	Habitat loss from current species ranges	Species extinctions (birds)	2100
(12)	GBO2	LUC, CC, N	IMAGE	Dose-response model (GLOBIO)	Species abundance changes	2050
(15)	IPCC SRES	CC	GCM (HadCM3)	Dynamic global vegetation models	Functional group range shifts (plants) and carbon sequestration	2100
<i>Freshwater</i>						
(22)	MA	Water use and CC	Water-GAP	Phenomenological model relating river discharge to fish species richness	Species extinctions (fishes)	2100
<i>Marine</i>						
(23)	GEO4, IAASTD	Fishing effort	Ecosim	Marine trophic model (Ecosim with Ecopath).	Functional group abundance changes and fish landings	2050
(43)	IPCC SRES	CC	GCMs (HadCM3, PCM)	Phenomenological model relating sea surface temperature to bleaching frequencies	Habitat loss of tropical corals	2100
(52)	IPCC SRES	CC	GCMs (GFDL CM 2.1)	Niche-based models.	Species range shifts (vertebrate and invertebrates)	2050

and habitat specificity [e.g., in (18), the highest extinction rates are 7% for broad habitat specificity versus 43% for narrow habitat specificity], emphasizing the need for research on these fundamental aspects of species ecology and their incorporation into global models (14). Third, a large fraction of variation between studies appears to arise from differences between modeling approaches [in particular, compare the two studies of global bird extinctions (6, 19)]. The few rigorous intermodel comparisons currently available have also found large differences in model sensitivity (20, 21).

Quantitative scenarios of global extinctions for freshwater and marine organisms are rare. One model for freshwater ecosystems, based on the relationship between fish diversity and river discharge, projects 4 to 22% (quartile range) fish extinctions by 2070 in about 30% of the world's rivers, because of reductions in river discharge from climate change and increasing water withdrawals (22). Models of global change impacts on marine organisms have focused on local extinctions, shifts in species distributions, and changes in abundance (23). The limited amount of extinction scenarios for aquatic ecosystems suggests that quantitative data for global extinctions models are still lacking (24), emphasizing the need for improved monitoring of marine and freshwater organisms.

Projections of species extinction rates are controversial because of methodological challenges and because of the lack of agreement with extinction patterns in the recent and distant past (25, 26). First, models project the fraction of species "committed to extinction," primarily resulting from decreases in range size, habitat area or, for freshwater taxa, river flow. However, the lag time between being "committed to extinction" and actually going extinct may range from decades to many millennia (13, 25), so future research must focus on quantifying these time lags as they constitute windows of opportunity for restoration efforts to prevent future extinctions. Evidence from recent and historical land-use change and the paleontological record suggests that many species can persist for long periods, by exploiting secondary habitats or by surviving in small populations (25, 27). This suggests that the realized extinction rates are likely to be lower and perhaps much lower than the "committed to extinction" rates shown in Fig. 2 and used in other comparisons of past and future extinction rates (28, 29). Second, complex interactions between global change factors are not accounted for in models, and these interactions could decrease or increase future extinction risks (25). These considerations and the range of projected terrestrial extinctions in Fig. 2 reflect general scientific agreement that uncurtailed rates of climate and land-use change will increase extinction risks but that the magnitude of these risks is still uncertain.

Field and laboratory experiments mimicking reductions in species and functional group diversity have shown that species loss at local scales

can have negative impacts on ecosystem services such as primary productivity, nutrient cycling, and invasion resistance (3). Extinctions of species that play dominant roles in ecosystem functioning, such as large predators and pollinators, could be extremely detrimental for ecosystem services (30). However, it has proved difficult to scale these studies up to regional or global scales.

Species Abundances and Community Structure

Models project declines in the population abundances of species in both marine and terrestrial systems (12, 23). Global scenarios for marine

future increases in landings, partially driven by fisheries subsidies, can only be achieved by intensifying pressure on groups that are not currently fished in large quantities, often at lower trophic levels, leading to a decline in the marine trophic index (23, 31). In contrast, reductions in fishing effort and destructive fishing practices such as trawling would allow rebuilding of a number of major stocks (31, 32).

For terrestrial systems, the GLOBIO model uses dose-response relationships to estimate changes in mean species abundance as a function of land-use change and other drivers (12). For

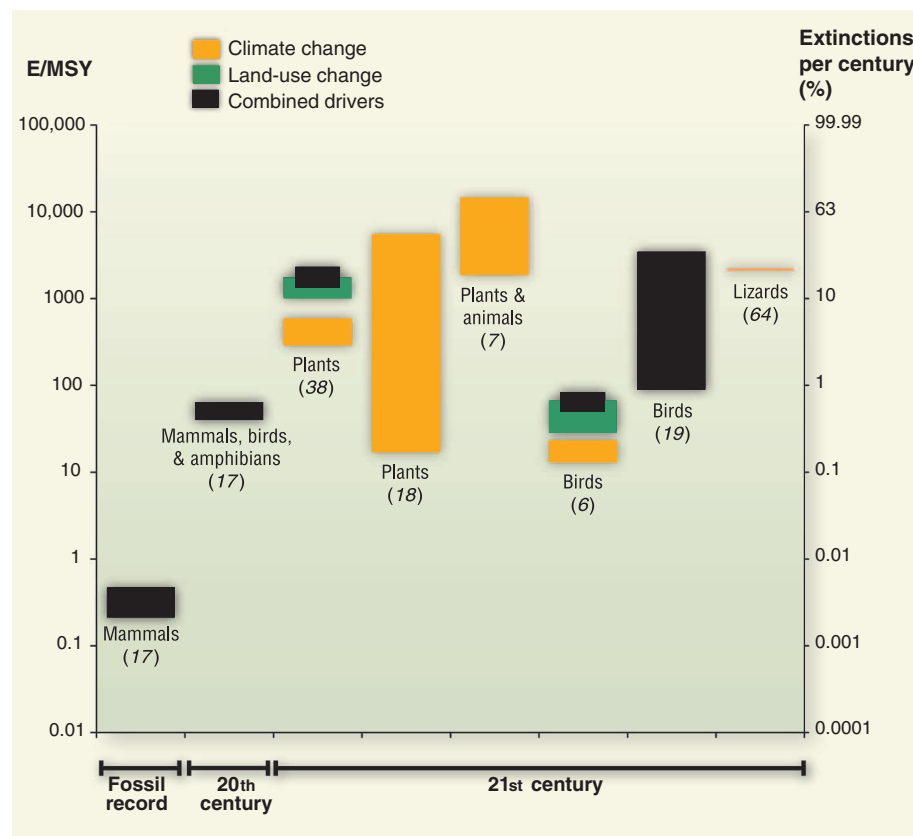


Fig. 2. Comparison of recent and distant past extinction rates with rates at which species are "committed to extinction" during the 21st century (63). E/MSY is number of extinctions per million species years; "Fossil record" refers to the extinction rate of mammals in the fossil record (17); "20th century" refers to documented extinctions in the 20th century—mammals (upper bound), birds, and amphibians (lower bound) (17); "21st century" refers to projections of species committed to extinction according to different global scenarios: vascular plants (38, 18), plants and animals (7), birds (6, 19), and lizards (64). Extinction rate caused by each driver and total extinction rates are discriminated, when possible.

fisheries are based on a marine trophic model, Ecosim with Ecopath, which tracks functional groups of species, including multiple groups of primary producers, invertebrates, and fish species (16). Model parameters are estimated from historic trends of biomass and catches. Future ecosystem dynamics are projected by optimizing fishing effort for a set of criteria, including profit, number of jobs, and ecosystem structure, with the weight given to each criterion depending on the scenario (23). The scenarios explored suggest that

instance, the model uses a matrix of changes in mean local species abundance after conversion between two land-use categories, derived from empirical studies. Scenarios developed using GLOBIO project a decline of 9 to 17% in mean species abundance by 2050 relative to 2000 (33, 34). The most favorable scenarios involve a doubling of protected areas to 20% of total land area or focusing on sustainability at all levels, with limited human population and consumption growth. The GLOBIO model has also been

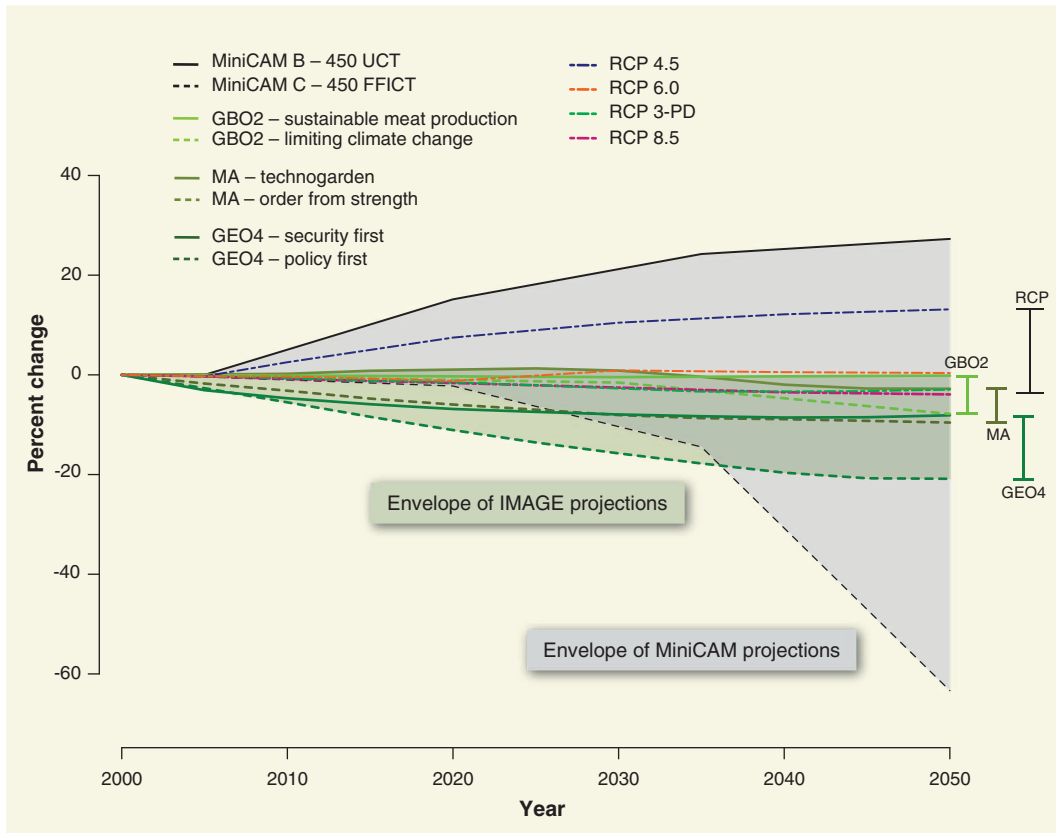


Fig. 3. Change in the extent of forests to 2050 in different global scenarios (63): MA scenarios (1), GBO2 scenarios (34), GEO4 scenarios (33), MiniCAM scenarios (39), and RCP scenarios for IPCC-AR5 (41). For each study, trajectories of the two most contrasting scenarios are shown. By 2050, the envelope of scenarios with the IMAGE model (MA, GBO2, GEO4) is narrower than the envelope of scenarios based on the MiniCAM model.

used to hindcast changes in mean species abundance from 1970 to 2000 (35). The modeled decline of 6% over this period is much smaller than the decline of 21% recorded by the Living Planet Index through direct observations of terrestrial species abundance (5). However, large differences in how these two indicators are calculated and potential biases in data in the Living Planet Index (36) and in the database of GLOBIO make direct comparison impossible and underscore the need to harmonize model and data indices (35).

Trade-offs between provisioning services and regulating services are apparent in both marine and terrestrial biodiversity scenarios and can be a consequence of changes in community structure. For instance, increases in fish provisioning are achieved at the cost of changes in the food web structure with potential impacts on the regulation of trophic cascades (37), and often at the cost of sacrificing the long-term sustainability of the service (32). Similarly, in Mediterranean ecosystems, modification of forest composition to favor rapid-growth species may lead to decreased fire resilience (35).

Habitat Loss and Degradation

Habitat loss and degradation in terrestrial ecosystems cover a wide range of alteration of natural and seminatural ecosystems by human activities.

Arguably, the conversion of forest to agricultural systems has been the most important of these habitat changes. In most land-use scenarios, global forest area declines slightly over the next few decades (Fig. 3), resulting from extensive deforestation in tropical forests and subtropical woodlands, which is partially offset by increased forest cover in the Northern Hemisphere (33, 34, 38). Therefore, in terms of impacts on biodiversity, the overall picture is worse than the global forest projections indicate, as the habitat losses in the tropics cannot be directly compensated by forest habitat gains in temperate regions, and some of the forest gains in both regions are due to the expansion of species-poor plantations.

A striking characteristic of some studies is that large within-study divergences in socioeconomic development pathways lead to relatively small differences in the projected global forest cover (Fig. 3), as well as other measures of global biodiversity change (12). The most recent studies, i.e., the Intergovernmental Panel on Climate Change Representative Concentration Pathways (IPCC RCP) (2) scenarios and Wise *et al.* (39), include more favorable trajectories, suggesting that the opportunities for habitat recovery may have been previously underestimated. In particular, Wise *et al.* (39) foresee large increases in global forest cover if global carbon taxes were to include all sources

and sinks of carbon, thereby favoring protection of forests and improved agricultural efficiency. However, they also project massive deforestation if carbon taxes were to focus on fossil fuels only, stimulating massive dependence on bioenergy. This study and other recent global scenarios (40) emphasize the positive or negative impact that climate mitigation could have on biodiversity depending on how it is implemented. The ongoing land-use harmonization activity for IPCC Assessment Report 5, which connects land-use historical data together with future scenario data from multiple Integrated Assessment Models into a single consistent, spatially gridded set of land-use change scenarios, will open new opportunities for exploring the impacts of a broad range of possible land-use trajectories on biodiversity and for enhancing the collaboration between the climate, socioeconomic, and biodiversity communities (41).

Climate change is projected to cause major changes in marine habitats, through increased water temperature, ocean acidification, and expansion of oxygen minimum zones (42). Tropical corals are vulnerable to climate change because increases in sea surface temperature of 1°C for more than 8 weeks can lead to severe coral bleaching, with the breakdown of the endosymbiosis between corals and zooxanthellae [(43); but see (44)]. Phenomenological models applied to climate change projections foresee that severe tropical coral bleaching may occur on average every 2 years by 2050 (43). In addition, ocean acidification reduces the availability of carbonate for calcification, slowing the growth of corals, and along with bleaching and other stressors, is projected to lead to widespread degradation of coral reefs and the ecosystem services they provide such as fisheries, storm surge protection, and income from tourism (45).

In freshwater ecosystems, modeling of habitat degradation has focused on the abiotic components of ecosystems, such as river discharge and nutrient loads, and how those changes will directly affect ecosystem services such as water provisioning and regulation of water quality (1, 46). In some cases, the same drivers affecting ecosystem services may also affect biodiversity. For example, scenario studies project increased water use as a consequence of population growth and rising water demands by agriculture and industry (33), and increased eutrophication due to agriculture and urban pollution (46). This will lead to

both water provisioning shortages and declines in biodiversity (13).

Shifts in the Distribution of Species and Biomes

DGVMs project large shifts in the distribution of terrestrial biomes, with required velocities to accommodate temperature change reaching more than 1 km year^{-1} in some biomes (47, 48). These shifts are expected to cause the rearrangement of ecosystems, including the creation of novel communities (49). For instance, the northern limit of boreal forests is projected to move further north into the arctic tundra, whereas the southern limit will experience dieback, giving way to temperate conifer and mixed forest (15, 47). DGVM projections are in qualitative agreement with the paleontological record, which indicates that climate change has resulted in large shifts in the distribution of vegetation types in the past. However, there is uncertainty in the extent of biome changes simulated by DGVMs, even in analyses using common climate scenarios, with some global vegetation models projecting modest shifts and little vegetation dieback and others projecting large-scale biome shifts over much of the globe during the 21st century, underscoring the pressing need to benchmark models against data (15).

DGVMs provide a powerful means to explore the relationship between ecosystem services and shifts in the distribution of biomes or functional groups of plants because vegetation type plays a dominant role in controlling terrestrial provisioning services, as well as supporting and regulating services. Recent simulations with DGVMs suggest that the Amazon forest may reach a tipping point due to a combination of deforestation, climate change, and fire, leading to drier conditions and an irreversible shift to savannah-like vegetation (50). If pushed beyond this point, the Amazonian forest could release large quantities of carbon into the atmosphere and modify rainfall patterns over large areas of South and southern North America (50).

Bioclimatic models of the ranges of marine organisms also suggest poleward shifts because of climate change (47). Average speeds for demersal species may exceed 4 km year^{-1} in certain regions (Fig. 4A), consistent with recent trends observed in the North Sea for widespread thermal specialists (51). Projected shifts for pelagic species are foreseen to be more rapid than for demersal species (Fig. 4B), owing to the

higher motility of pelagic species and larger changes in ocean conditions in the surface layer. Furthermore, rates of shift can be more than double in a high-range climate change scenario (A1B) compared to a low-range scenario (committed climate change experiment) (52), suggesting that limiting greenhouse gas emissions will allow more time for species to adapt. The capacity of freshwater species to move polewards in response to climate change will be more limited owing to the linear nature of many freshwater ecosystems. This problem will be particularly acute in river basins with an east-west configuration (35). Species may also respond to warming by migration to higher elevations in terrestrial systems (19) and greater depths in marine systems (53).

Given the rapidly growing use of bioclimatic models for decision support, such as studies of the impacts of climate change on future costs and efficiency of networks of protected areas (54) and development of adaptive forestry management schemes (55), it is important that model projec-

tions are accurate. Bioclimatic models can, in some cases, predict the direction of range contractions or expansions (56) and population increases or declines (57) observed in the last few decades. However, insufficient treatment of key mechanisms, such as migration, biotic interactions, and interactions between drivers such as climate and land use, still limits the accuracy of future range projections from bioclimatic models (14).

Challenges in Improving Biodiversity Scenarios

Reducing uncertainty within and among model projections is urgent. More attention must be paid to evaluating model projections with indicators that allow comparisons between models and between models and data. Key components of this effort will be the development of comprehensive biodiversity monitoring through efforts such as the Global Biodiversity Observation Network or GEO BON (58), and the harmonization of the biodiversity indicators used by the data and scenarios communities.

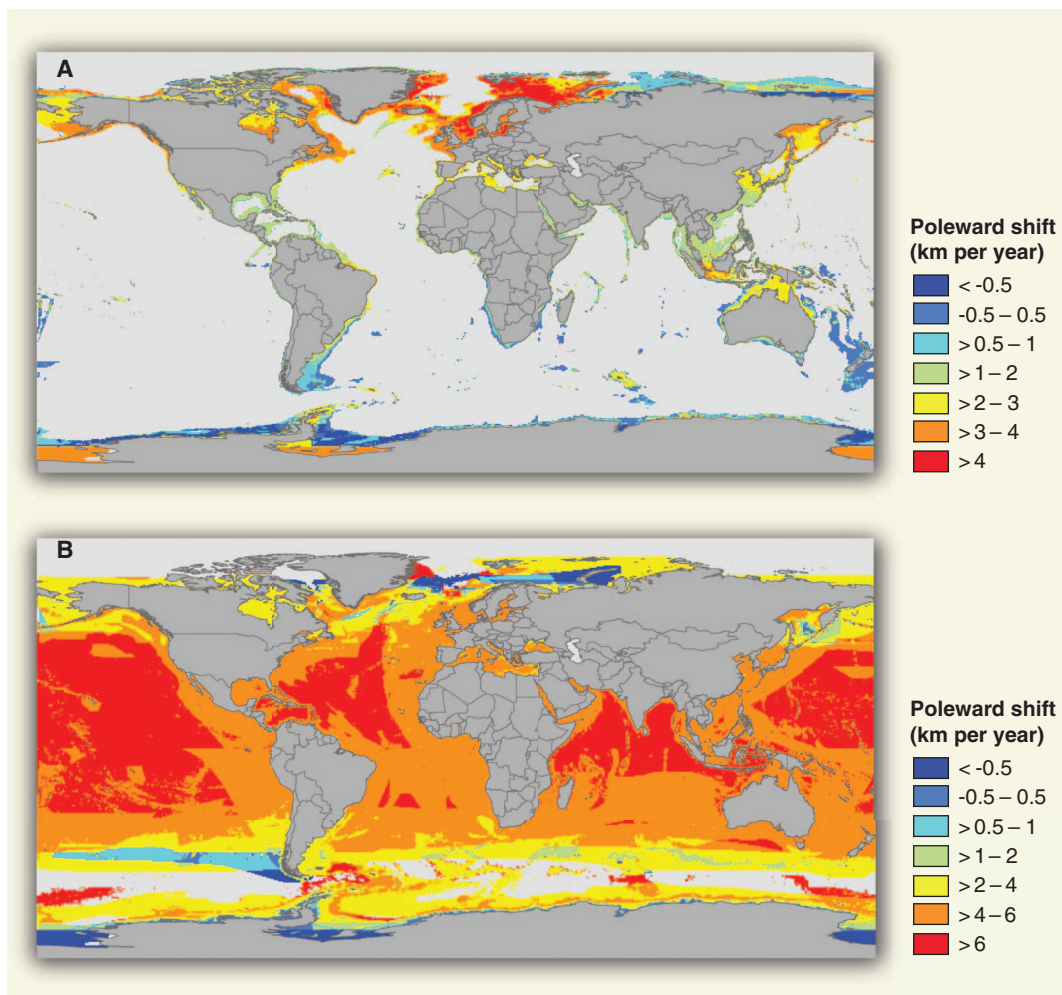


Fig. 4. Projected rate of range shifts in marine organisms caused by climate change from 2005 to 2050 (52, 63). (A) Latitudinal shift of demersal species (excluding areas >2000 m in depth because of undersampling of the deep-sea region). (B) Latitudinal shift of pelagic species. The projections are based on bioclimatic envelope models for 1066 species of fish and invertebrates, under IPCC SRES A1B. For each map cell, the mean shift of the range centroids of the species currently present in that cell is given.

The importance of the drivers of biodiversity change differs across realms, with land-use change being a dominant driver in terrestrial systems, and overexploitation in marine systems, while climate change is ubiquitous across realms (28). Available models reflect these differences, but fail to account for the full set of major drivers of future biodiversity change—for instance, the lack of global models of the impact of dams and pollution on freshwater biodiversity. Modeling climate change impacts on biodiversity is currently tractable and popular, in part because a wide range of climate scenarios and bioclimatic envelope modeling tools are readily available, but it is vital to develop models of other important drivers and their interactions (59). This will require the development of mechanistic models linking changes in land use, pollution levels, and biotic competition (e.g., invasive species) to population dynamics of individual species through changes in life-history parameters such as survival and dispersal, using techniques that are scalable across space and across species assemblages (10, 14). This approach has recently been explored to assess how interactions between life history and disturbance regime mediate species extinction risk under climate change (60).

To better inform policy, scenarios must move beyond illustrating the potential impacts of global change on biodiversity toward more integrated approaches that account for the feedbacks that link environmental drivers, biodiversity, ecosystem services, and socioeconomic dynamics. Current global biodiversity models rarely relate estimates of biodiversity loss to ecosystem services, infrequently explore policy options [but see (12, 23)], and do not account for the feedbacks from changes in biodiversity and ecosystem services to societal response (Fig. 1, dashed arrows). Introducing complex feedbacks to biodiversity scenarios will require moving away from the relatively linear, noninteractive relationships between the social and natural sciences (Fig. 1, thick downward-pointing arrows) toward a more interactive, interdisciplinary association (61).

The likely imminent launch of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) opens an opportunity to develop a major effort to improve and evaluate biodiversity scenarios. Improved biodiversity models will strengthen the role of scenarios in testing policies to minimize the impacts of human activities on biodiversity and maximize the ecosystem services provided by biodiversity. Hence, scenarios should play a large role in IPBES and in helping to achieve the targets set in the new strategic plan of the Convention on Biological Diversity (62).

References and Notes

1. J. Alcamo, D. van Vuuren, C. Ringer, in *Ecosystems and Human Well-Being: Scenarios*, S. R. Carpenter, L. P. Prabhu, E. M. Bennet, M. B. Zurek, Eds. (Island Press, Washington, DC, 2005), pp. 147–172.
2. R. H. Moss *et al.*, *Nature* **463**, 747 (2010).
3. P. Balvanera *et al.*, *Ecol. Lett.* **9**, 1146 (2006).
4. S. Díaz, J. Fargione, F. S. Chapin III, D. Tilman, *PLoS Biol.* **4**, e277 (2006).
5. S. H. M. Butchart *et al.*, *Science* **328**, 1164 (2010).
6. W. Jetz, D. S. Wilcove, A. P. Dobson, *PLoS Biol.* **5**, e157 (2007).
7. C. D. Thomas *et al.*, *Nature* **427**, 145 (2004).
8. A. Balmford, R. E. Green, M. Jenkins, *Trends Ecol. Evol.* **18**, 326 (2003).
9. P. Kareiva *et al.*, in *Ecosystems and Human Well-Being: Scenarios*, R. J. Scholes, R. Hassan, Eds. (Island Press, Washington, DC, 2005), pp. 73–115.
10. H. M. Pereira, G. C. Daily, *Ecology* **87**, 1877 (2006).
11. R. K. Heikkinen *et al.*, *Prog. Phys. Geogr.* **30**, 751 (2006).
12. R. Alkamade *et al.*, *Ecosystems* (N.Y.) **12**, 374 (2009).
13. O. E. Sala *et al.*, in *Ecosystems and Human Well-Being: Scenarios*, S. R. Carpenter, L. P. Prabhu, E. M. Bennet, M. Zurek, Eds. (Island Press, Washington, DC, 2005), pp. 375–408.
14. W. Thuiller *et al.*, *Perspect. Plant Ecol. Evol. Syst.* **9**, 137 (2008).
15. S. Sitch *et al.*, *Glob. Change Biol.* **14**, 2015 (2008).
16. V. Christensen, C. J. Walters, *Ecol. Modell.* **172**, 109 (2004).
17. G. M. Mace, H. Masundire, J. E. M. Baillie, in *Ecosystems and Human-Well Being: Current State and Trends*, B. Scholes, R. Hassan, Eds. (Island Press, Washington, DC, 2005), pp. 77–122.
18. J. R. Malcolm, C. Liu, R. P. Neilson, L. Hansen, L. Hannah, *Conserv. Biol.* **20**, 538 (2006).
19. C. H. Sekercioglu, S. H. Schneider, J. P. Fay, S. R. Loarie, *Conserv. Biol.* **22**, 140 (2008).
20. R. G. Pearson *et al.*, *J. Biogeogr.* **33**, 1704 (2006).
21. X. Morin, W. Thuiller, *Ecology* **90**, 1301 (2009).
22. M. A. Xenopoulos *et al.*, *Glob. Change Biol.* **11**, 1557 (2005).
23. J. Alder, S. Guénette, J. Beblow, W. Cheung, V. Christensen, *Ecosystem-Based Global Fishing Policy Scenarios* (Fisheries Centre Research Reports 15(7), University of British Columbia, 2007).
24. N. K. Dulvy, Y. Sadovy, J. Reynolds, *Fish Fish.* **4**, 25 (2003).
25. N. E. Stork *et al.*, *Conserv. Biol.* **23**, 1438 (2009).
26. D. B. Botkin *et al.*, *Bioscience* **57**, 227 (2007).
27. K. J. Willis, S. A. Bhagwat, *Science* **326**, 806 (2009).
28. A. Duraipappah *et al.*, *Ecosystems and Human Well-Being: Biodiversity Synthesis* (World Resources Institute, Washington, DC, 2005).
29. S. L. Pimm, G. J. Russell, J. L. Gittleman, T. M. Brooks, *Science* **269**, 347 (1995).
30. M. A. Aizen, L. A. Garibaldi, S. A. Cunningham, A. M. Klein, *Ann. Bot. (London)* **103**, 1579 (2009).
31. D. Pauly *et al.*, *Science* **302**, 1359 (2003).
32. B. Worm *et al.*, *Science* **325**, 578 (2009).
33. UNEP, *Global Environment Outlook 4* (United Nations Environment Programme, Nairobi, Kenya, 2007).
34. B. ten Brink *et al.*, *Cross-Roads of Planet Earth's Life. Exploring Means to Meet the 2010-Biodiversity Target* (MNP report 55050001/2006, Netherlands Environmental Agency, Bilthoven, 2006).
35. P. W. Leadley *et al.*, *Biodiversity Scenarios: Projections of 21st Century Change in Biodiversity and Associated Ecosystem Services* (Secretariat of the Convention on Biological Diversity, Montreal, 2010).
36. H. M. Pereira, H. David Cooper, *Trends Ecol. Evol.* **21**, 123 (2006).
37. M. Casini *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 197 (2009).
38. D. van Vuuren, O. Sala, H. M. Pereira, *Ecol. Soc.* **11**, 25 (2006).
39. M. Wise *et al.*, *Science* **324**, 1183 (2009).
40. A. M. Thomson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* (2010).
41. G. C. Hurtt *et al.*, Harmonization of global land-use scenarios for the period 1500–2100 for the 5th IPCC Assessment. *iLEAPS Newsletter* 7, June 2009.
42. O. Hoegh-Guldberg, J. F. Bruno, *Science* **328**, 1523 (2010).
43. S. D. Donner, W. J. Skirving, C. M. Little, M. Oppenheimer, O. Hoegh-Guldberg, *Glob. Change Biol.* **11**, 2251 (2005).
44. J. Maynard, A. Baird, M. Pratchett, *Coral Reefs* **27**, 745 (2008).
45. O. Hoegh-Guldberg *et al.*, *Science* **318**, 1737 (2007).
46. A. F. Bouwman, G. Van Drecht, J. M. Knoop, A. H. W. Beusen, C. R. Meinardi, *Global Biogeochem. Cycles* **19**, GB1002 (2005).
47. A. Fischlin *et al.*, in *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the IPCC*, M. Parry, O. Canziani, J. Palutikof, P. van der Linden, C. Hanson, Eds. (Cambridge Univ. Press, Cambridge, 2007), pp. 211–272.
48. S. R. Loarie *et al.*, *Nature* **462**, 1052 (2009).
49. J. W. Williams, S. T. Jackson, J. E. Kutzbach, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 5738 (2007).
50. D. C. Nepstad, C. M. Stickler, B. S. Filho, F. Merry, *Philos. Trans. R. Soc. B* **363**, 1737 (2008).
51. A. L. Perry, P. J. Low, J. R. Ellis, J. D. Reynolds, *Science* **308**, 1912 (2005).
52. W. W. L. Cheung *et al.*, *Fish Fish.* **10**, 235 (2009).
53. N. K. Dulvy *et al.*, *J. Appl. Ecol.* **45**, 1029 (2008).
54. L. Hannah *et al.*, *Front. Ecol. Environ.* **5**, 131 (2007).
55. M. S. Mbogga, X. Wang, A. Hamann, *J. Appl. Ecol.* **47**, 731 (2010).
56. M. B. Araújo, R. G. Pearson, W. Thuiller, M. Erhard, *Glob. Change Biol.* **11**, 1504 (2005).
57. R. E. Green *et al.*, *Biol. Lett.* **4**, 599 (2008).
58. R. J. Scholes *et al.*, *Science* **321**, 1044 (2008).
59. B. W. Brook, N. S. Sodhi, C. J. A. Bradshaw, *Trends Ecol. Evol.* **23**, 453 (2008).
60. D. A. Keith *et al.*, *Biol. Lett.* **4**, 560 (2008).
61. S. R. Carpenter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 1305 (2009).
62. UNEP/CBD/SP/2009/2: *Revision and Updating of the CBD Strategic Plan: Possible Outline and Elements of the New Strategic Plan* (2009); www.cbd.int/sp/sp2010p.
63. Materials and methods are available as supporting material on Science online.
64. B. Sinervo *et al.*, *Science* **328**, 894 (2010).
65. This work was developed in the context of a scenarios synthesis coordinated by DIVERSITAS and UNEP World Conservation Monitoring Centre for the Secretariat of the Convention on Biological Diversity, with financial support from the Department of the Environment, Food and Rural Affairs of the UK, the European Commission, and UNEP. We thank L. Simpson for organizing a workshop. H.M.P. was supported in part by grant PTDC/AMB/73901/2006 from Fundação para a Ciência e Tecnologia.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1196624/DC1
Materials and Methods

Table S1

References and Notes

19 August 2010; accepted 13 October 2010

Published online 26 October 2010;

10.1126/science.1196624

Slow Earthquakes Linked Along Dip in the Nankai Subduction Zone

Hitoshi Hirose,^{1*} Youichi Asano,¹ Kazushige Obara,^{2,1} Takeshi Kimura,¹ Takanori Matsuzawa,¹ Sachiko Tanaka,¹ Takuto Maeda^{3,2}

Slow earthquakes, as defined by anomalously low rupture velocity, reflect a different slip regime than fast faulting of regular earthquakes. In the Cascadia and Nankai subduction zones, episodic tremor and slip (ETS) occur in the transition zone between locked and steady sliding zones along the plate interface (1–3). ETS, composed of concurrent non-volcanic tremor and a short-term slow slip event (SSE), is characterized by migration of the sources together along the strike direction of the subducting slab for several days to weeks (2–4). In the Bungo channel region at the western margin of the Nankai megathrust rupture zone (5), long-term SSEs, which last for several months, recur just up dip from the ETS zone about every 6 years (6, 7) and activate ETS (7, 8). In addition, shallow very-low-frequency earthquakes (VLFs) occur in some areas along the Nankai trough (9). However, the connection between shallow VLFs and other slow earthquakes remains unknown. We present new observations of temporally correlated occurrences of a long-term SSE, shallow VLFs, and tremor.

Shallow VLFs were identified from July 2003, and tremor was recorded from January 2001 through the recurrence of the long-term SSEs in 2003 and 2009–2010 (Fig. 1A) (10). The Global Positioning System (GPS) displacement time series of each SSE is characterized by a slow onset and an ac-

celeration phase in the middle of an episode. The up dip tremor activity in the Bungo channel (Fig. 1B) shows a rapid increase in tremor count that corresponds to the acceleration phase in the GPS record during the two SSEs (Fig. 1A), and the tremor activity gradually decreases as the SSE de-

celerates. In contrast, the downdip tremor (Fig. 1B) exhibits steady activity with an almost constant rate throughout the observation period (8). At the same time, the shallow VLFE activity to the south (Fig. 1B) abruptly begins almost simultaneously with the acceleration phase of the SSEs (Fig. 1A). The VLFE activity is of shorter duration than the SSEs and the up dip tremor activity, becoming quiet well before the end of the SSEs.

The epicenters of the up dip tremor are covered by the slip area of the SSE, whereas most of the downdip tremor is located outside of the slip area (Fig. 1B). This indicates that the contrast in the tremor activities is controlled by the extent of the SSE slip area. A similar relation between SSE slip and shallow VLFEs is suggested; that is, the SSE slip area may extend through the region of no measurable slip to the shallower VLFE source area and activate the VLFEs.

If slow slip connects the deep ETS zone and the shallow VLFE area in the dip direction, then the source area of slow earthquakes might act as a barrier to nearby megathrust rupturing because the slow slip area repeatedly releases the accumulated strain in an interseismic period of the megathrust events. Moreover, the slip area adjoins the megathrust rupture zone, suggesting that the repeating aseismic slip can modulate the stress buildup on the rupture zone. This indicates the importance of monitoring slow earthquakes as proxies for the stress modulation process.

References and Notes

1. K. Obara, *Science* **296**, 1679 (2002).
2. G. Rogers, H. Dragert, *Science* **300**, 1942 (2003); 10.1126/science.1084783.
3. K. Obara, H. Hirose, F. Yamamizu, K. Kasahara, *Geophys. Res. Lett.* **31**, L23602 (2004).
4. Y. Ito, K. Obara, K. Shiomi, S. Sekine, H. Hirose, *Science* **315**, 503 (2007); 10.1126/science.1134454.
5. T. Sagiya, W. Thatcher, *J. Geophys. Res.* **104**, 1111 (1999).
6. H. Hirose, K. Hirahara, F. Kimata, N. Fujii, S. Miyazaki, *Geophys. Res. Lett.* **26**, 3237 (1999).
7. H. Hirose, K. Obara, *Earth Planets Space* **57**, 961 (2005).
8. K. Obara, S. Tanaka, T. Maeda, T. Matsuzawa, *Geophys. Res. Lett.* **37**, L13306 (2010).
9. K. Obara, Y. Ito, *Earth Planets Space* **57**, 321 (2005).
10. Materials and methods are available as supporting material on Science Online.
11. GEONET, operated by the Geospatial Information Authority of Japan, is gratefully acknowledged.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1502/DC1

Materials and Methods

References

30 August 2010; accepted 12 November 2010
10.1126/science.1197102

¹National Research Institute for Earth Science and Disaster Prevention, 3-1 Tennodai, Tsukuba, Ibaraki 305-0006, Japan.

²Earthquake Research Institute, University of Tokyo, 1-1-1 Yayoi, Bunkyo, Tokyo 113-0032, Japan. ³Center for Integrated Disaster Information Research, Interfaculty Initiative in Information Studies, University of Tokyo, 1-1-1 Yayoi, Bunkyo, Tokyo 113-0032, Japan.

*To whom correspondence should be addressed. E-mail: hirose@bosai.go.jp

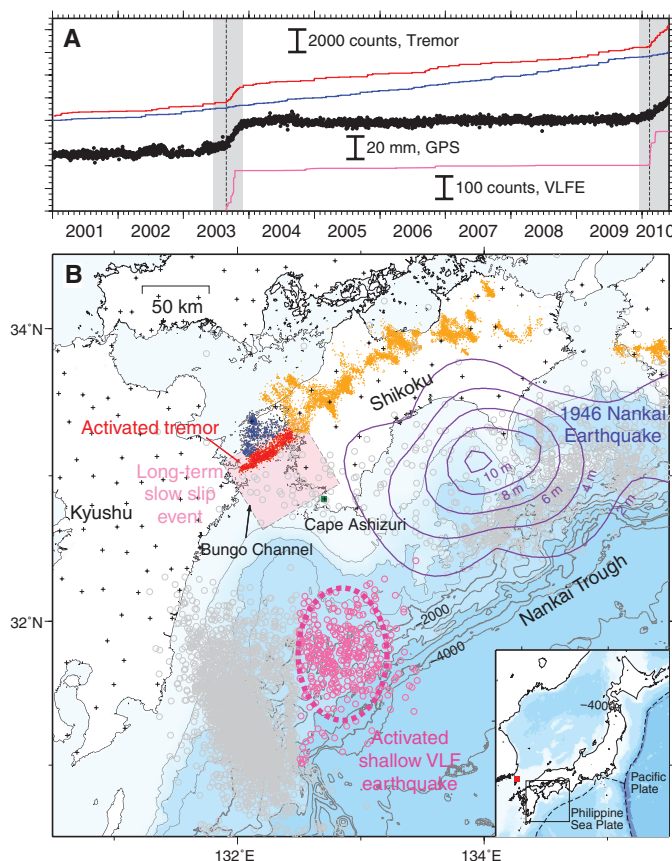


Fig. 1. (A) Time series of the cumulative number of shallow VLFs (pink line) to the south, off Cape Ashizuri [pink circles in (B)], cumulative number of tremor sources in the downdip (blue line) and up dip (red line) regions in the Bungo channel [blue and red dots in (B)], and (black dots) detrended GPS displacement record (east component) at Ohtsuki [green square in (B)] with respect to Kamitsushima [red square in (B) inset]. The shaded regions indicate the approximate durations of the two long-term SSEs that occurred in 2003 and 2009–2010. The dashed lines denote the beginning of the VLFE activity off Cape Ashizuri. (B) Map of tremor epicenters (orange, blue, and red dots), VLF epicenters (gray and pink circles), and the 2010 Bungo channel long-term SSE fault (pink rectangle) in southwest Japan. Purple contours show the slip distribution of the 1946 Nankai earthquake (5). Gray contours show ocean depths.

The Impact of Conservation on the Status of the World's Vertebrates

Michael Hoffmann,^{1,2*} Craig Hilton-Taylor,³ Ariadne Angulo,^{4,5} Monika Böhm,⁶ Thomas M. Brooks,^{7,8,9} Stuart H. M. Butchart,¹⁰ Kent E. Carpenter,^{2,5,11} Janice Chanson,^{5,12} Ben Collen,⁶ Neil A. Cox,^{5,13} William R. T. Darwall,³ Nicholas K. Dulvy,¹⁴ Lucy R. Harrison,¹⁴ Vineet Katariya,³ Caroline M. Pollock,³ Suhel Quader,¹⁵ Nadia I. Richman,⁶ Ana S. L. Rodrigues,¹⁶ Marcelo F. Tognelli,^{5,13,17} Jean-Christophe Vié,⁵ John M. Aguiar,¹⁸ David J. Allen,³ Gerald R. Allen,¹⁹ Giovanni Amori,²⁰ Natalia B. Ananjeva,²¹ Franco Andreone,²² Paul Andrew,²³ Aida Luz Aquino Ortiz,²⁴ Jonathan E. M. Baillie,²⁵ Ricardo Baldi,^{26,27} Ben D. Bell,²⁸ S. D. Biju,²⁹ Jeremy P. Bird,³⁰ Patricia Black-Decima,³¹ J. Julian Blanc,³² Federico Bolaños,³³ Wilmar Bolívar-G.,³⁴ Ian J. Burfield,¹⁰ James A. Burton,^{35,36} David R. Capper,³⁷ Fernando Castro,³⁸ Gianluca Catullo,³⁹ Rachel D. Cavanagh,⁴⁰ Alan Channing,⁴¹ Ning Labbish Chao,^{42,43,44} Anna M. Chenery,⁴⁵ Federica Chiozza,⁴⁶ Viola Clausnitzer,⁴⁷ Nigel J. Collar,¹⁰ Leah C. Collett,³ Bruce B. Collette,⁴⁸ Claudia F. Cortez Fernandez,⁴⁹ Matthew T. Craig,⁵⁰ Michael J. Crosby,¹⁰ Neil Cumberlidge,⁵¹ Annabelle Cuttelod,³ Andrew E. Derocher,⁵² Arvin C. Diesmos,⁵³ John S. Donaldson,⁵⁴ J. W. Duckworth,⁵⁵ Guy Dutson,⁵⁶ S. K. Dutta,⁵⁷ Richard H. Emslie,⁵⁸ Aljos Farjon,⁵⁹ Sarah Fowler,⁶⁰ Jörg Freyhof,⁶¹ David L. Garshelis,⁶² Justin Gerlach,⁶³ David J. Gower,⁶⁴ Tandora D. Grant,⁶⁵ Geoffrey A. Hammerson,⁶⁶ Richard B. Harris,⁶⁷ Lawrence R. Heaney,⁶⁸ S. Blair Hedges,⁶⁹ Jean-Marc Hero,⁷⁰ Baz Hughes,⁷¹ Syed Ainul Hussain,⁷² Javier Icochea M.,⁷³ Robert F. Inger,⁶⁸ Nobuo Ishii,⁷⁴ Djoko T. Iskandar,⁷⁵ Richard K. B. Jenkins,^{76,77,78} Yoshio Kaneko,⁷⁹ Maurice Kottelat,^{80,81} Kit M. Kovacs,⁸² Sergius L. Kuzmin,⁸³ Enrique La Marca,⁸⁴ John F. Lamoreux,^{5,85} Michael W. N. Lau,⁸⁶ Esteban O. Lavilla,⁸⁷ Kristin Leus,⁸⁸ Rebecca L. Lewison,⁸⁹ Gabriela Lichtenstein,⁹⁰ Suzanne R. Livingstone,⁹¹ Vimoksalehi Lukoschek,^{92,93} David P. Mallon,⁹⁴ Philip J. K. McGowan,⁹⁵ Anna McIvor,⁹⁶ Patricia D. Moehlan,⁹⁷ Sanjay Molur,⁹⁸ Antonio Muñoz Alonso,⁹⁹ John A. Musick,¹⁰⁰ Kristin Nowell,¹⁰¹ Ronald A. Nussbaum,¹⁰² Wanda Olech,¹⁰³ Nikolay L. Orlov,²¹ Theodore J. Papenfuss,¹⁰⁴ Gabriela Parra-Olea,¹⁰⁵ William F. Perrin,¹⁰⁶ Beth A. Polidoro,^{5,11} Mohammad Pourkazemi,¹⁰⁷ Paul A. Racey,¹⁰⁸ James S. Ragle,⁵ Mala Ram,⁶ Galen Rathbun,¹⁰⁹ Robert P. Reynolds,¹¹⁰ Anders G. J. Rhodin,¹¹¹ Stephen J. Richards,^{112,113} Lily O. Rodríguez,¹¹⁴ Santiago R. Ron,¹¹⁵ Carlo Rondinini,⁴⁶ Anthony B. Rylands,² Yvonne Sadovy de Mitcheson,^{116,117} Jonnell C. Sanciangco,^{5,11} Kate L. Sanders,¹¹⁸ Georgina Santos-Barrera,¹¹⁹ Jan Schipper,¹²⁰ Caryn Self-Sullivan,^{121,122} Yichuan Shi,³ Alan Shoemaker,¹²³ Frederick T. Short,¹²⁴ Claudio Sillero-Zubiri,¹²⁵ Débora L. Silvano,¹²⁶ Kevin G. Smith,³ Andrew T. Smith,¹²⁷ Jos Snoeks,^{128,129} Alison J. Stattersfield,¹⁰ Andrew J. Symes,¹⁰ Andrew B. Taber,¹³⁰ Bibhab K. Talukdar,¹³¹ Helen J. Temple,¹³² Rob Timmins,¹³³ Joseph A. Tobias,¹³⁴ Katerina Tsytulina,¹³⁵ Denis Tweddle,¹³⁶ Carmen Ubeda,¹³⁷ Sarah V. Valenti,⁶⁰ Peter Paul van Dijk,² Liza M. Veiga,^{138,139} Alberto Veloso,¹⁴⁰ David C. Wege,¹⁰ Mark Wilkinson,⁶⁴ Elizabeth A. Williamson,¹⁴¹ Feng Xie,¹⁴² Bruce E. Young,⁷ H. Resit Akçakaya,¹⁴³ Leon Bennun,¹⁰ Tim M. Blackburn,⁶ Luigi Boitani,⁴⁶ Holly T. Dublin,^{144,145} Gustavo A. B. da Fonseca,^{146,147} Claude Gascon,² Thomas E. Lacher Jr.,¹⁸ Georgina M. Mace,¹⁴⁸ Susan A. Mainka,¹⁴⁹ Jeffery A. McNeely,¹⁴⁹ Russell A. Mittermeier,^{2,149} Gordon McGregor Reid,¹⁵⁰ Jon Paul Rodriguez,¹⁵¹ Andrew A. Rosenberg,² Michael J. Samways,¹⁵² Jane Smart,¹⁴⁹ Bruce A. Stein,¹⁵³ Simon N. Stuart^{1,2,154,155}

Using data for 25,780 species categorized on the International Union for Conservation of Nature Red List, we present an assessment of the status of the world's vertebrates. One-fifth of species are classified as Threatened, and we show that this figure is increasing: On average, 52 species of mammals, birds, and amphibians move one category closer to extinction each year. However, this overall pattern conceals the impact of conservation successes, and we show that the rate of deterioration would have been at least one-fifth again as much in the absence of these. Nonetheless, current conservation efforts remain insufficient to offset the main drivers of biodiversity loss in these groups: agricultural expansion, logging, overexploitation, and invasive alien species.

In the past four decades, individual populations of many species have undergone declines and many habitats have suffered losses of

original cover (1, 2) through anthropogenic activity. These losses are manifested in species extinction rates that exceed normal background rates

by two to three orders of magnitude (3), with substantial detrimental societal and economic consequences (4). In response to this crisis, 193 parties to the Convention on Biological Diversity (CBD; adopted 1992) agreed “to achieve by 2010 a significant reduction of the current rate of biodiversity loss at the global, regional, and national level as a contribution to poverty alleviation and to the benefit of all life on Earth” (5). That the target has not been met was borne out by empirical testing against 31 cross-disciplinary indicators developed within the CBD framework itself (1). However, this does not mean that conservation efforts have been ineffective. Conservation actions have helped to prevent extinctions (6, 7) and improve population trajectories (8), but there has been limited assessment of the overall impact of ongoing efforts in reducing losses in biodiversity (9, 10). Here, we assess the overall status of the world's vertebrates, determine temporal trajectories of extinction risk for three vertebrate classes, and estimate the degree to which conservation actions have reduced biodiversity loss.

Described vertebrates include 5498 mammals, 10,027 birds, 9084 reptiles, 6638 amphibians, and 31,327 fishes (table S1). Vertebrates are found at nearly all elevations and depths, occupy most major habitat types, and display remarkable variation in body size and life history. Although they constitute just 3% of known species, vertebrates play vital roles in ecosystems (11) and have great cultural importance (12). Under the auspices of the International Union for Conservation of Nature (IUCN) Species Survival Commission, we compiled data on the taxonomy, distribution, population trend, major threats, conservation measures, and threat status for 25,780 vertebrate species, including all mammals, birds, amphibians, cartilaginous fishes, and statistically

¹IUCN SSC Species Survival Commission, c/o United Nations Environment Programme World Conservation Monitoring Centre, 219 Huntingdon Road, Cambridge CB3 0DL, UK. ²Conservation International, 2011 Crystal Drive, Arlington, VA 22202, USA. ³Species Programme, IUCN, 219c Huntingdon Road, Cambridge CB3 0DL, UK. ⁴IUCN–CI Biodiversity Assessment Unit, c/o P.O. Box 19004, 360 A Bloor Street W., Toronto, Ontario M5S 1X1, Canada. ⁵Species Programme, IUCN, Rue Mauverney 28, 1196, Gland, Switzerland. ⁶Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK. ⁷NatureServe, 1101 Wilson Boulevard, Arlington, VA 22209, USA. ⁸World Agroforestry Center (ICRAF), University of the Philippines Los Baños, Laguna 4031, Philippines. ⁹School of Geography and Environmental Studies, University of Tasmania, Hobart, Tasmania 7001, Australia. ¹⁰BirdLife International, Wellbrook Court, Girton Road, Cambridge CB3 0NA, UK. ¹¹Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529, USA. ¹²IUCN–CI Biodiversity Assessment Unit, c/o 130 Weatherall Road, Cheltenham 3192, Victoria, Australia. ¹³IUCN–CI Biodiversity Assessment Unit, Conservation International, 2011 Crystal Drive Ste 500, Arlington, VA 22202, USA. ¹⁴IUCN Shark Specialist Group, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada. ¹⁵National Centre for Biological Sciences, Tata Institute of Fundamental Research, GKVK Campus, Bellary Road, Bangalore 560 065, India. ¹⁶Centre d'Ecologie Fonctionnelle et Evolutive, CNRS UMR5175, 1919 Route de Mende, 34293 Montpellier,

- France.¹⁷ADIZA-CONICET, CCT-Mendoza, CC 507, 5500 Mendoza, Argentina.¹⁸Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843, USA.¹⁹Western Australian Museum, Locked Bag 49, Welshpool DC, Perth, Western Australia 6986, Australia.²⁰CNR-Institute for Ecosystem Studies, Viale dell'Università 32, 00185 Rome, Italy.²¹Zoological Institute, Russian Academy of Sciences, 199034 St. Petersburg, Universitetskaya nab.1, Russia.²²Museo Regionale di Scienze Naturali, Via G. Giolitti, 36, I-10123 Torino, Italy.²³Taronga Conservation Society Australia, Taronga Zoo, P.O. Box 20, Mosman 2088, Sydney, Australia.²⁴Martin Barrios 2230 c/ Pizarro; Barrio Republicano, Asunción, Paraguay.²⁵Zoological Society of London, Regent's Park, London, NW1 4RY, UK.²⁶Unidad de Investigación Ecología Terrestre, Centro Nacional Patagónico-CONICET, Boulevard Brown 2915, 9120 Puerto Madryn, Argentina.²⁷Patagonian and Andean Steppe Program, Wildlife Conservation Society, Boulevard Brown 2915, 9120 Puerto Madryn, Argentina.²⁸Centre for Biodiversity & Restoration Ecology, School of Biological Sciences, Victoria University of Wellington, P.O. Box 600, Wellington 6140, New Zealand.²⁹Systematics Lab, School of Environmental Studies, University of Delhi, Delhi 110 007, India.³⁰Center for Biodiversity and Biosecurity Studies, Pacific Institute for Sustainable Development, Jalan Bumi Nyir 101, Manado, North Sulawesi, Indonesia.³¹Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, Miguel Lillo 205, 4000 SM de Tucumán, Tucumán, Argentina.³²P.O. Box 47074, Nairobi 00100, Kenya.³³Escuela de Biología, Universidad de Costa Rica, San Pedro, 11501-2060 San José, Costa Rica.³⁴Sección de Zoología, Departamento de Biología, Facultad de Ciencias Naturales y Exactas, Universidad del Valle, Calle 13, No. 100-00, Cali, Colombia.³⁵Earthwatch Institute, 256 Banbury Road, Oxford OX2 7DE, UK.³⁶Veterinary Biomedical Sciences, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Summerhall, Edinburgh EH9 1QH, Scotland, UK.³⁷47B Lewisham Hill, London SE13 7PL, UK.³⁸Laboratorio de Herpetología, Universidad del Valle, Carrera 51, No. 8H-15, Cali, Colombia.³⁹WWF Italy-Species Office, Via Po 25/c 00198 Rome, Italy.⁴⁰British Antarctic Survey, High Cross, Madingley Road, Cambridge CB3 0ET, UK.⁴¹Biodiversity and Conservation Biology Department, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa.⁴²Bio-Amazônia Conservation International, 1295 William Street, Baltimore, MD 21230, USA.⁴³Universidade Federal do Amazonas, Depto Ciências Pesqueiras, Manaus, AM 60700, Brazil.⁴⁴National Museum of Marine Biology and aquarium, 2 Houwan Road, Checheng, Pingtung 944, Taiwan, R.O.C.⁴⁵United Nations Environment Programme World Conservation Monitoring Centre, 219 Huntingdon Road, Cambridge CB3 0DL, UK.⁴⁶Department of Animal and Human Biology, Sapienza Università di Roma, Viale dell'Università 32, 00185 Roma, Italy.⁴⁷Senckenberg Museum of Natural History Goerlitz, PF 300 154, 02806 Goerlitz, Germany.⁴⁸National Marine Fisheries Service Systematics Laboratory, National Museum of Natural History, MRC-0153, Smithsonian Institution, Washington, DC 20013, USA.⁴⁹Av. Busch, Edificio Girasoles 2, Piso 5, Depto 7, La Paz, Bolivia.⁵⁰Department of Marine Sciences, University of Puerto Rico, P.O. Box 9000, Mayagüez, PR 00681, USA.⁵¹Department of Biology, Northern Michigan University, Marquette, MI 49855, USA.⁵²Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada.⁵³Herpetology Section, Zoology Division, National Museum of the Philippines, Padre Burgos Avenue, Ermita 1000, Manila, Philippines.⁵⁴South African National Biodiversity Institute, KRC, Private Bag X7, Claremont 7735, South Africa.⁵⁵P.O. Box 5573, Vientiane, Lao PDR.⁵⁶c/o Birds Australia, 60 Leicester Street, Carlton, Victoria 3053, Australia.⁵⁷North Orissa University, Sriram Chandra Vihar, Takatpur, Baripada 757003, Dist: Mayurbhanj, Orissa, India.⁵⁸IUCN SSC African Rhino Specialist Group, Box 1212, Hilton 3245, South Africa.⁵⁹Herbarium, Library, Art & Archives, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK.⁶⁰NatureBureau, 36 Kingfisher Court, Hambridge Road, Newbury RG14 5SJ, UK.⁶¹Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany.⁶²Minnesota Department of Natural Resources, Grand Rapids, MN 55744, USA.⁶³Nature Protection Trust of Seychelles, 133 Cherry Hinton Road, Cambridge CB1 7BX, UK.⁶⁴Department of Zoology, Natural History Museum, London SW7 5BD, UK.⁶⁵San Diego Zoo Institute for Conservation Research, 15600 San Pasqual Valley Road, Escondido, CA 92027, USA.⁶⁶NatureServe, 746 Middlepoint Road, Port Townsend, WA 98368, USA.⁶⁷Department of Ecosystem and Conservation Science, University of Montana, Missoula, MT 59812, USA.⁶⁸Field Museum of Natural History, Chicago, IL 60605, USA.⁶⁹Department of Biology, Pennsylvania State University, University Park, PA 16802, USA.⁷⁰Environmental Futures Centre, School of Environment, Griffith University, Gold Coast campus, Queensland, 4222, Australia.⁷¹Wildfowl & Wetlands Trust, Slimbridge, Glos GL2 7BT, UK.⁷²Wildlife Institute of India, Post Box #18, Dehra Dun, 248001 Uttarakhand, India.⁷³Calle Arica 371, Dpto U-2, Miraflores, Lima 18, Perú.⁷⁴School of Arts and Sciences, Tokyo Woman's Christian University, Zempukuiji 2-6-1, Suginami-ku, Tokyo 167-8585, Japan.⁷⁵School of Life Sciences and Technology, Institut Teknologi Bandung, 10, Jalan Ganesa, Bandung 40132, Indonesia.⁷⁶Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, University of Kent, Canterbury, Kent CT2 7NR, UK.⁷⁷School of the Environment and Natural Resources, Bangor University, Bangor LL57 2UW, UK.⁷⁸Madagasikara Voakajy, B.P. 5181, Antananarivo (101), Madagascar.⁷⁹Iwate Prefectural University, Sugo 152-52, Takizawa, Iwate 020-0193, Japan.⁸⁰Route de la Baroche 12, 2952 Cornol, Switzerland.⁸¹Raffles Museum of Biodiversity Research, National University of Singapore, Department of Biological Sciences, 6 Science Drive 2, #03-01, 117546, Singapore.⁸²Norwegian Polar Institute, 9296 Tromsø, Norway.⁸³Institute of Ecology and Evolution, Russian Academy of Sciences, Leninsky Prospect, 33, Moscow 119071, Russia.⁸⁴Laboratorio de Biogeografía, Escuela de Geografía, Universidad de Los Andes, Mérida 5101, Venezuela.⁸⁵IUCN Species Programme, c/o 406 Randolph Hill Road, Randolph, NH 03593, USA.⁸⁶Kadoorie Farm & Botanic Garden, Lam Kam Road, Tai Po, New Territories, Hong Kong SAR.⁸⁷Instituto de Herpetología, Fundación Miguel Lillo-CONICET, Miguel Lillo 251, 4000 SM de Tucumán, Tucumán, Argentina.⁸⁸Conservation Breeding Specialist Group-Eurpam Regional Office, p/a Annuntienstraat 6, 2170 Merksem, Belgium.⁸⁹Biology Department, San Diego State University, San Diego, CA 92182, USA.⁹⁰Instituto Nacional de Antropología y Pensamiento Latinoamericano, 3 de Febrero 1378, 1426 Buenos Aires, Argentina.⁹¹Ecology and Evolutionary Biology, Faculty of Biomedical & Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ, Scotland, UK.⁹²Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, USA.⁹³ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Queensland, 4811, Australia.⁹⁴Department of Biology, Chemistry and Health Science, Manchester Metropolitan University, Manchester M1 5GD, UK.⁹⁵World Pheasant Association, Newcastle University Biology Field Station, Close House Estate, Heddon on the Wall, Newcastle upon Tyne NE15 0HT, UK.⁹⁶115 Suez Road, Cambridge CB1 3QD, UK.⁹⁷Wildlife Trust Alliance, Box 2031, Arusha, Tanzania.⁹⁸Zoo Outreach Organisation, 9A Lal Bahadur Colony, Peelamedu, Coimbatore, Tamil Nadu 641004, India.⁹⁹El Colegio de la Frontera Sur, Apartado postal 63, Carretera Panamericana y Periférico sur s/n Col. María Auxiliadora, 29290, San Cristóbal de las Casas, Chiapas, México.¹⁰⁰Virginia Institute of Marine Science, Gloucester Point, VA 23062, USA.¹⁰¹CAT, P.O. Box 332, Cape Neddick, ME 03902, USA.¹⁰²Division of Reptiles and Amphibians, Museum of Zoology, University of Michigan, Ann Arbor, MI 48109, USA.¹⁰³Warsaw University of Life Sciences, Ciszewskiego 8, 02-786 Warsaw, Poland.¹⁰⁴Museum of Vertebrate Zoology, University of California, Berkeley, CA 94720, USA.¹⁰⁵Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, 04510 Ciudad Universitaria, México.¹⁰⁶Southwest Fisheries Science Center, National Marine Fisheries Service, NOAA, 3333 North Torrey Pines Court, La Jolla, CA 92037, USA.¹⁰⁷International Sturgeon Research Institute, P.O. Box 41635-3464, Rasht, Iran.¹⁰⁸Centre for Ecology and Conservation, University of Exeter in Cornwall, Penryn TR10 9EZ, UK.¹⁰⁹Department of Ornithology and Mammalogy, California Academy of Sciences (San Francisco), c/o P.O. Box 202, Cambria, CA 93428, USA.¹¹⁰USGS Patuxent Wildlife Research Center, MRC 111, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, Washington, DC 20013, USA.¹¹¹Chelonian Research Foundation, 168 Goodrich Street, Lunenburg, MA 01462, USA.¹¹²Herpetology Department, South Australian Museum, North Terrace, Adelaide, South Australia 5000, Australia.¹¹³Rapid Assessment Program, Conservation International, P.O. Box 1024, Atherton, Queensland 4883, Australia.¹¹⁴German Technical Cooperation (GTZ) GmbH, Pasaje Bernardo Alcedo N° 150, piso 4, El Olivar, San Isidro, Lima 27, Perú.¹¹⁵Museo de Zoología, Escuela de Biología, Pontificia Universidad Católica del Ecuador, Av. 12 de Octubre y Veintimilla, Quito, Ecuador.¹¹⁶School of Biological Sciences, University of Hong Kong, Pok Fu Lam Road, Hong Kong SAR.¹¹⁷Society for the Conservation of Reef Fish Aggregations, 9888 Carroll Centre Road, Suite 102, San Diego, CA 92126, USA.¹¹⁸School of Earth and Environmental Sciences, Darling Building, University of Adelaide, North Terrace, Adelaide 5005, Australia.¹¹⁹Departamento de Biología Evolutiva, Facultad de Ciencias, Universidad Nacional Autónoma de México, Circuito Exterior S/N, 04510, Ciudad Universitaria, México.¹²⁰Big Island Invasive Species Committee, Pacific Cooperative Studies Unit, University of Hawaii, 23 East Kawili Street, Hilo, HI 96720, USA.¹²¹Sirenian International, 200 Stonewall Drive, Fredericksburg, VA 22401, USA.¹²²Department of Biology, P.O. Box 8042, Georgia Southern University, Statesboro, GA 30460, USA.¹²³IUCN SSC Tapir Specialist Group, 330 Shareditch Road, Columbia, SC 29210, USA.¹²⁴Department of Natural Resources and the Environment, University of New Hampshire, Jackson Estuarine Laboratory, Durham, NH 03824, USA.¹²⁵Wildlife Conservation Research Unit, Department of Zoology, University of Oxford, Recanati-Kaplan Centre, Tubney House, Tubney OX13 5QL, UK.¹²⁶Laboratório de Zoologia, Universidade Católica de Brasília, Campus I-Q.S. 07 Lote 01 EPTC-Taguatinga-DF, 71966-700, Brazil.¹²⁷School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA.¹²⁸Royal Museum for Central Africa, Ichthyology, Leuvensesteenweg 13, B-3080 Tervuren, Belgium.¹²⁹Katholieke Universiteit Leuven, Laboratory of Animal Diversity and Systematics, Charles Deberiotstraat 32, B-3000 Leuven, Belgium.¹³⁰Center for International Forestry Research, Jalan CIFOR, Situ Gede, Bogor Barat 16115, Indonesia.¹³¹Aaryak and International Rhino Foundation, 50 Samarwoy Path (Survey), Post Office Beltola, Guwahati-781 028, Assam, India.¹³²The Biodiversity Consultancy Ltd., 4 Woodend, Trumpington, Cambridge CB2 9LJ, UK.¹³³2313 Willard Avenue, Madison, WI 53704, USA.¹³⁴Edward Grey Institute, Department of Zoology, University of Oxford, Oxford OX1 3PS, UK.¹³⁵Vertebrate Research Division, National Institute of Biological Resources, Environmental Research Complex, Gyoungseo-dong, Seo-gu, Incheon 404-708, South Korea.¹³⁶South African Institute for Aquatic Biodiversity, P/Bag 1015, Grahamstown, 6140, South Africa.¹³⁷Departamento de Zoología, Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, Quintral 1250, 8400 Bariloche, Argentina.¹³⁸Emilio Goeldi Museum, Av. Perimetral, 1901, Belém, Pará 66017-970, Brazil.¹³⁹Federal University of Pará, Rua Augusto Corrêa, 01, Belém, Pará 66075-110, Brazil.¹⁴⁰Departamento de Ciências Ecológicas, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Casilla 6553, Santiago, Chile.¹⁴¹Department of Psychology, University of Stirling, Stirling FK9 4LA, Scotland, UK.¹⁴²Chengdu Institute of Biology, the Chinese Academy of Sciences, Chengdu, 610041, P.R. China.¹⁴³Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY 11794, USA.¹⁴⁴IUCN SSC, African Elephant Specialist Group, c/o IUCN ESARO, P.O. Box 68200, Nairobi 00200, Kenya.¹⁴⁵Wildlife Conservation Society, 2300 Southern Boulevard, Bronx, NY 10460, USA.¹⁴⁶Global Environment Facility, 1818 H Street NW, G 6-602, Washington, DC 20433, USA.¹⁴⁷Department of Zoology, Federal University of Minas Gerais, 31270-901, Belo Horizonte, Brazil.¹⁴⁸Centre for Population Biology, Imperial College London, Silwood Park, Ascot, Berks SL5 7PY, UK.¹⁴⁹IUCN, 28 Rue Mauverney, CH-1196 Gland, Switzerland.¹⁵⁰North of England Zoological Society, Chester Zoo, Upton-by-Chester, Chester CH2 1LH, UK.¹⁵¹Centro de Ecología, Instituto Venezolano de Investigaciones Científicas, Apartado 20632, Caracas 1020-A, Venezuela, and Provita, Apartado 47552, Caracas 1041-A, Venezuela.¹⁵²Department of Conservation Ecology and Entomology, Stellenbosch University, P/Bag X1, Matieland 7602, South Africa.¹⁵³National Wildlife Federation, 901 E Street NW, Suite 400, Washington, DC 20004, USA.¹⁵⁴Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, UK.¹⁵⁵Al Ain Wildlife Park & Resort, P.O. Box 45553, Abu Dhabi, United Arab Emirates.

*To whom correspondence should be addressed. E-mail: mike.hoffmann@iucn.org

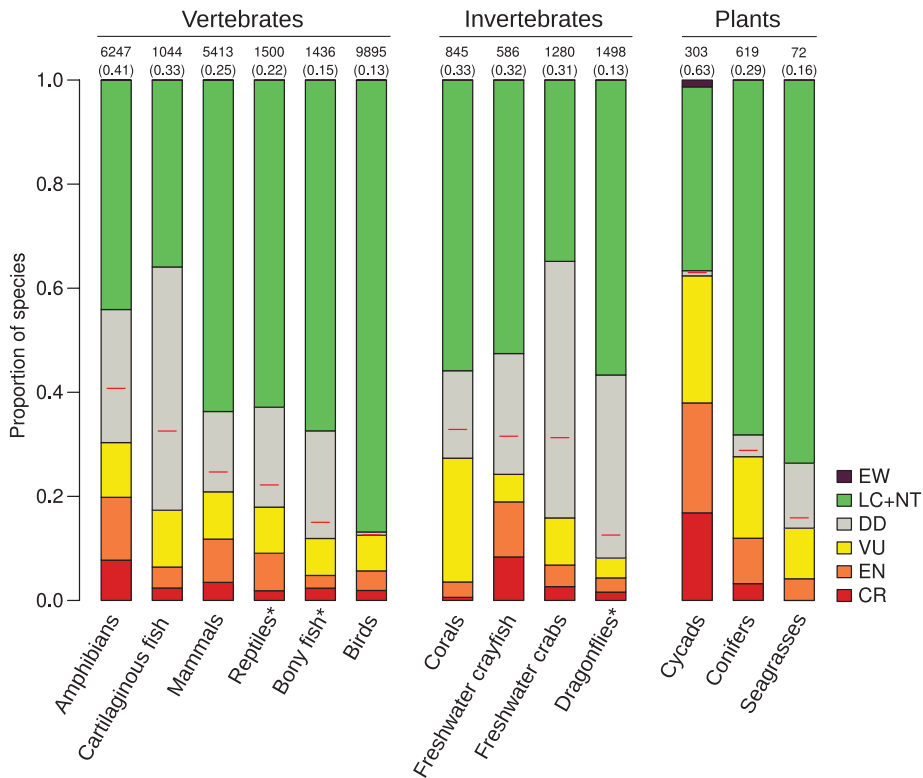


Fig. 1. The proportion of vertebrate species in different Red List categories compared with completely (or representatively) assessed invertebrate and plant taxa on the 2010 IUCN Red List (15). EW, Extinct in the Wild; CR, Critically Endangered; EN, Endangered; VU, Vulnerable; NT, Near Threatened; LC, Least Concern; DD, Data Deficient. Extinct species are excluded. Taxa are ordered according to the estimated percentage (shown by horizontal red lines and given in parentheses at tops of bars) of extant species considered Threatened if Data Deficient species are Threatened in the same proportion as data-sufficient species. Numbers above the bars represent numbers of extant species assessed in the group; asterisks indicate those groups in which estimates are derived from a randomized sampling approach.

representative samples of reptiles and bony fishes [~1500 species each (13)].

The IUCN Red List is the widely accepted standard for assessing species' global risk of extinction according to established quantitative criteria (14). Species are categorized in one of eight categories of extinction risk, with those in the categories Critically Endangered, Endangered, or Vulnerable classified as Threatened. Assessments are designed to be transparent, objective, and consultative, increasingly facilitated through workshops and Web-based open-access systems. All data are made freely available for consultation (15) and can therefore be challenged and improved upon as part of an iterative process toward ensuring repeatable assessments over time.

Status, trends, and threats. Almost one-fifth of extant vertebrate species are classified as Threatened, ranging from 13% of birds to 41% of amphibians, which is broadly comparable with the range observed in the few invertebrate and plant taxa completely or representatively assessed to date (Fig. 1 and table S2). When we incorporate the uncertainty that Data Deficient species (those with insufficient information for determining risk of extinction) introduce, the proportion of all vertebrate species classified as Threatened is between 16% and 33% (midpoint = 19%; table S3). [Further details of the data and assumptions behind these values are provided in (16) and tables S2 and S3.] Threatened vertebrates occur mainly in tropical regions (Fig. 2), and these concentrations are generally disproportionately high even when accounting for their high overall species

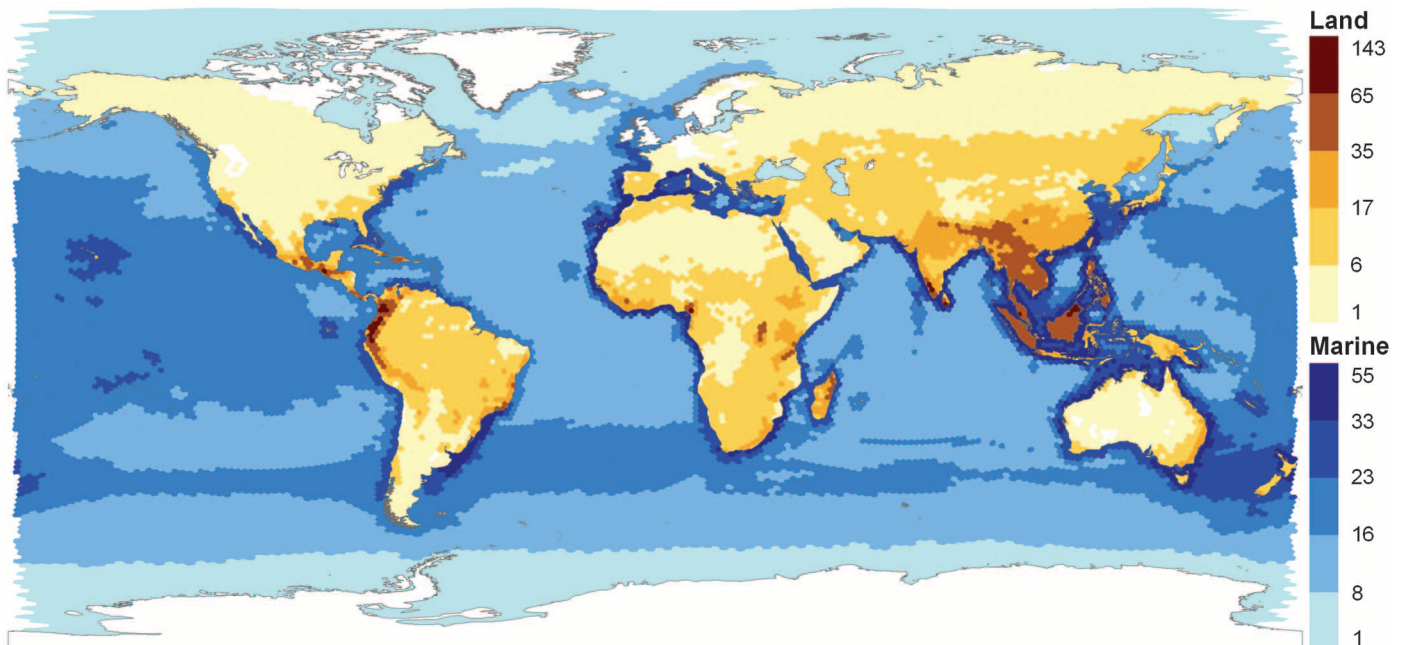


Fig. 2. Global patterns of threat, for land (terrestrial and freshwater, in brown) and marine (in blue) vertebrates, based on the number of globally Threatened species in total.

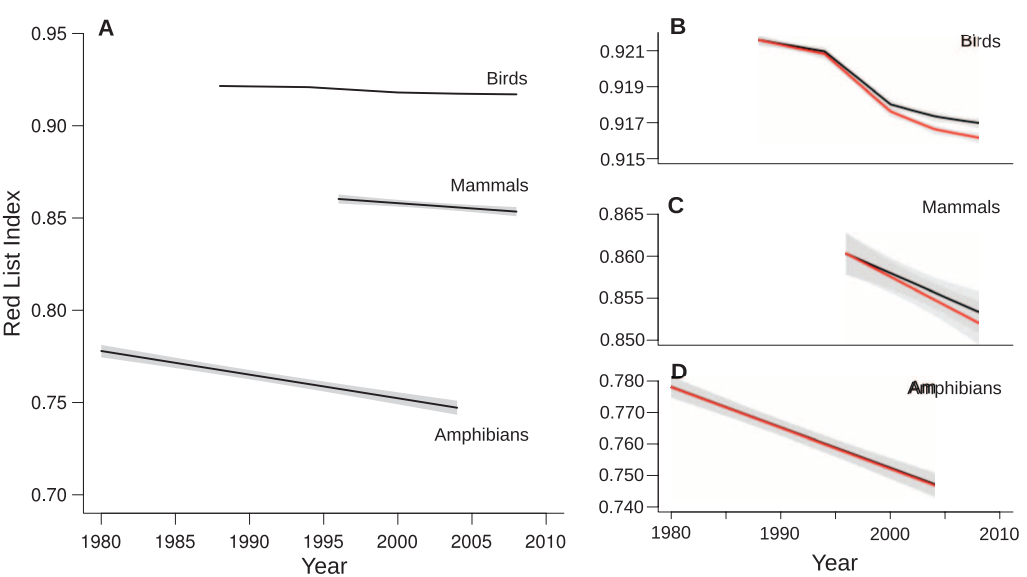
richness (fig. S4, A and B). These patterns highlight regions where large numbers of species with restricted distributions (17) coincide with intensive direct and indirect anthropogenic pressures, such as deforestation (18) and fisheries (19). To investigate temporal trends in extinction risk of vertebrates, we used the IUCN Red List Index (RLI) methodology (20) that has been

Table 1. Net number of species qualifying for revised IUCN Red List categories between assessments owing to genuine improvement or deterioration in status, for birds (1988 to 2008), mammals (1996 to 2008), and amphibians (1980 to 2004). Category abbreviations are as for Fig. 1; CR(PE/PEW) denotes Critically Endangered (Possibly Extinct or Possibly Extinct in the Wild). CR excludes PE/PEW. Species undergoing an improvement (i.e., moving from a higher to a lower category of threat) are indicated by "+"; species de-

teriorating in status (i.e., moving from a lower to a higher category of threat) are indicated by "-". Species changing categories for nongenuine reasons, such as improved knowledge or revised taxonomy, are excluded. In the case of birds, for which multiple assessments have been undertaken, values in parentheses correspond to the sum of all changes between consecutive assessments; the same species may therefore contribute to the table more than once [see (16)].

		Red List category at end of period							
		CR			CR	EN	VU	NT	LC
		EX	EW	(PE/PEW)					
Birds	EX		0	0	0	0	0	0	0
	EW	0		0	+1 (+1)	0	0	0	0
	CR (PE/PEW)	0	0		0	0	0	0	0
	CR	-2 (-2)	-2 (-2)	-7 (-7)		+16 (+19)	+1 (+3)	0	0
	EN	0	0	0	-22 (-27)		+4 (+5)	0	0
	VU	0	0	0	-10 (-11)	-34 (-41)		+9 (+10)	0 (+1)
	NT	0	0	0	-4 (-4)	-5 (-2)	-40 (-47)		+1 (+1)
	LC	0	0	0	-1 (0)	-5 (-4)	-5 (-5)	-78 (-81)	
	Mammals	EX	0	0	0	0	0	0	0
	EW	0		0	+1	+1	0	0	0
Mammals	CR (PE/PEW)	0	0		0	0	0	0	0
	CR	0	-1	-3		+3	+2	0	0
	EN	0	0	0	-31		+3	+1	0
	VU	0	0	0	-2	-39		+5	+1
	NT	0	0	0	-1	-4	-47		+7
	LC	0	0	0	0	-2	-2	-39	
Amphibians	EX		0	0	0	0	0	0	0
	EW	0		0	0	0	0	0	0
	CR (PE/PEW)	-2	0		0	0	0	0	0
	CR	-3	-1	-34		0	+2	0	0
	EN	-2	0	-42	-77		0	+2	0
	VU	-2	0	-19	-51	-45		0	0
	NT	0	0	0	-7	-18	-32		0
	LC	0	0	0	-3	-8	-20	-92	

Fig. 3. (A) Trends in the Red List Index (RLI) for the world's birds, mammals, and amphibians. (B to D) Observed change in the RLI for each group (black) compared with RLI trends that would be expected if species that underwent an improvement in status due to conservation action had undergone no change (red). The difference is attributable to conservation. An RLI value of 1 equates to all species being Least Concern; an RLI value of 0 equates to all species being Extinct. Improvements in species conservation status lead to increases in the RLI; deteriorations lead to declines. A downward trend in the RLI value means that the net expected rate of species extinctions is increasing. Shading shows 95% confidence intervals. Note: RLI scales for (B), (C), and (D) vary.



adopted for reporting against global targets (1, 2). We calculated the change in RLI for birds (1988, 1994, 2000, 2004, and 2008), mammals (1996 and 2008), and amphibians (1980 and 2004); global trend data are not yet available for other vertebrate groups, although regional indices have been developed (21). The RLI methodology is explained in detail in (16), but in summary the index is an aggregated measure of extinction risk calculated from the Red List categories of all assessed species in a taxon, excluding Data Deficient species. Changes in the RLI over time result from species changing categories between assessments (Table 1). Only real improvements or deteriorations in status (termed “genuine” changes) are considered; re-categorizations attributable to improved knowledge, taxonomy, or criteria change (“nongenuine” changes) are excluded (22). Accordingly, the RLI is calculated only after earlier Red List categorizations are retrospectively corrected using current information and taxonomy, to ensure that the same species are considered throughout and that only genuine changes are included. For example, the greater red musk shrew (*Crocidura flavescens*) was classified as Vulnerable in 1996 and as Least Concern in 2008; however, current evidence indicates that the species was also Least Concern in 1996, and the apparent improvement is therefore a nongenuine change. In contrast, Hose’s broadbill (*Calypotomena hosii*)

was one of 72 bird species to deteriorate one Red List category between 1994 and 2000, from Least Concern to Near Threatened, mainly because of accelerating habitat loss in the Sundaic lowlands in the 1990s. Such a deterioration in a species’ conservation status leads to a decline in the RLI (corresponding to increased aggregated extinction risk); an improvement would lead to an increase in the RLI.

Temporal trajectories reveal declining RLIs for all three taxa. Among birds, the RLI (Fig. 3A) showed that their status deteriorated from 1988 to 2008, with index values declining by 0.49%, an average of 0.02% per year (table S4). For mammals, the RLI declined by 0.8% from 1996 to 2008, a faster rate (0.07% per year) than for birds. Proportionally, amphibians were more threatened than either birds or mammals; RLI values declined 3.4% from 1980 to 2004 (0.14% per year). Although the absolute and proportional declines in RLIs for each taxonomic group were small, these represent considerable biodiversity losses. For example, the deterioration for amphibians was equivalent to 662 amphibian species each moving one Red List category closer to extinction over the assessment period. The deteriorations for birds and mammals equate to 223 and 156 species, respectively, deteriorating at least one category. On average, 52 species per year moved one Red List category closer to extinction from 1980 to

2008. Note that the RLI does not reflect ongoing population changes that are occurring too slowly to trigger change to different categories of threat. Other indicators based on vertebrate population sizes showed declines of 30% between 1970 and 2007 (1, 2, 22).

Global patterns of increase in overall extinction risk are most marked in Southeast Asia (Fig. 4 and figs. S5A and S6). It is known that the planting of perennial export crops (such as oil palm), commercial hardwood timber operations, agricultural conversion to rice paddies, and unsustainable hunting have been detrimental to species in the region (23), but here we show the accelerating rate at which these forces are driving change. In California, Central America, the tropical Andean regions of South America, and Australia, patterns have been driven mainly by the “enigmatic” deteriorations among amphibians (24), which have increasingly been linked to the infectious disease chytridiomycosis, caused by the presumed invasive fungal pathogen *Batrachochytrium dendrobatidis* (25). Almost 40 amphibians have deteriorated in status by three or more IUCN Red List categories between 1980 and 2004 (Table 1).

Although chytridiomycosis has been perhaps the most virulent threat affecting vertebrates to emerge in recent years, it is not the only novel cause of rapid declines. The toxic effects of the veterinary drug diclofenac on Asian vultures have

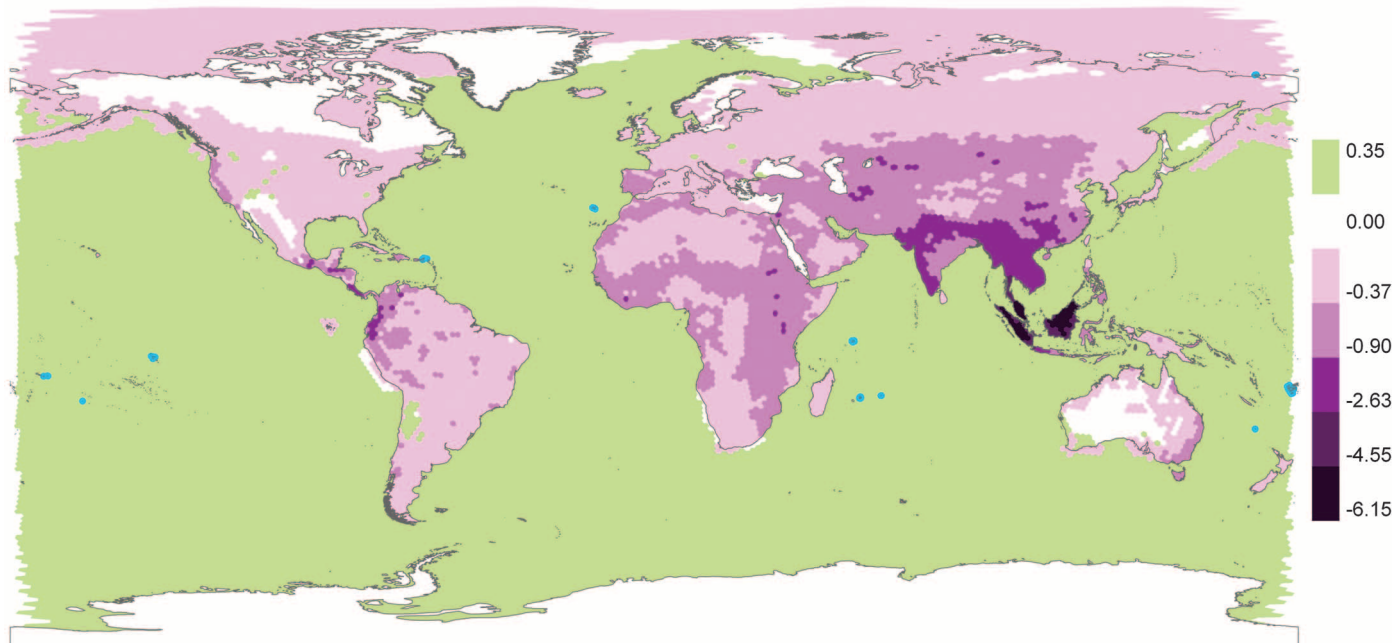


Fig. 4. Global patterns of net change in overall extinction risk across birds, mammals, and amphibians (for the periods plotted in Fig. 3) mapped as average number of genuine Red List category changes per cell per year. Purple corresponds to net deterioration (i.e., net increase in extinction risk) in that cell; green, net improvement (i.e., decrease in extinction risk); white, no change. The uniform pattern of improvement at sea is driven by improvements of migratory marine mammals with

cosmopolitan distributions (e.g., the humpback whale). Deteriorations on islands [e.g., the nightingale reed-warbler (*Acrocephalus luscinius*) in the Northern Mariana Islands] and improvements on islands [e.g., the Rarotonga monarch (*Pomarea dimidiata*) in the Cook Islands] are hard to discern; islands showing overall net improvements are shown in blue. Note that the intensity of improvements never matches the intensity of deteriorations.

caused estimated population declines exceeding 99% over the past two decades in certain *Gyps* species, and have resulted in three species moving from Near Threatened to Critically Endangered between 1994 and 2000. Numbers of Tasmanian devils (*Sarcophilus harrisii*) have fallen by more than 60% in the past 10 years because of the emergence of devil facial tumor disease (resulting in three step changes from Least Concern to Endangered). Climate change is not yet adequately captured by the IUCN Red List (26, 27) but has been directly implicated in the deteriorating status of several vertebrates and may interact with other threats to hasten extinction (28). However, there is no evidence of a parallel to the systemwide deteriorations documented for reef-building corals affected by bleaching events related to El Niño–Southern Oscillation occurrences (29).

Most deteriorations in status are reversible, but in 13% of cases they have resulted in extinction. Two bird species—the kamao (*Myadestes myadestinus*) from Hawaii and the Alaotra grebe (*Tachybaptus rufolavatus*) from Madagascar—became extinct between 1988 and 2008, and a further six Critically Endangered species have been flagged as “possibly extinct” during this period (Table 1 and table S5). At least nine amphibian species vanished during the two decades after 1980, including the golden toad (*Incilius periglenes*) from Costa Rica and both of Australia’s unique gastric-brooding frog species (genus *Rheobatrachus*); a further 95 became possibly extinct, 18 of them harlequin toads in the Neotropical genus *Atelopus* (23% of species). No mammals are listed as Extinct for the period 1996 to 2008, although the possible extinction of the Yangtze River dolphin (*Lipotes vexillifer*) would be the first megafauna vertebrate species extinction since the Caribbean monk seal in the 1950s (30).

Estimates of conservation success. These results support previous findings that the state of biodiversity continues to decline, despite increasing trends in responses such as protected areas coverage and adoption of national legislation (1, 2). Next, we asked whether conservation efforts have made any measurable contribution to reducing declines or improving the status of biodiversity.

The RLI trends reported here are derived from 928 cases of recategorization on the IUCN Red List (Table 1 and table S6), but not all of these refer to deteriorations. In 7% of cases (68/928), species underwent an improvement in status, all but four due to conservation action. For example, the Asian crested ibis (*Nipponia nippon*) changed from Critically Endangered in 1994 to Endangered in 2000 owing to protection of nesting trees, control of agrochemicals in rice fields, and prohibition of firearms; the four exceptions were improvements resulting from natural processes, such as unassisted habitat regeneration (tables S7 and S8). Nearly all of these improvements involved mammals

and birds, where the history of conservation extends farther back and where the bulk of species-focused conservation funding and attention is directed (31). Only four amphibian species underwent improvements, because the amphibian extinction crisis is such a new phenomenon and a plan for action has only recently been developed (32).

To estimate the impact of conservation successes, we compared the observed changes in the RLI with the RLI trends expected if all 64 species that underwent an improvement in status due to conservation action had not done so (16). Our explicit assumption is that in the absence of conservation, these species would have remained unchanged in their original category, although we note that this approach is conservative because it is likely that some would have deteriorated [in the sense of (6)]. The resulting difference between the two RLIs can be attributed to conservation. We show that the index would have declined by an additional 18% for both birds and mammals in the absence of conservation (Fig. 3, B and C, and table S4). There was little difference for amphibians (+1.4%; Fig. 3D) given the paucity of species improvements. For birds, conservation action reduced the decline in the RLI from 0.58% to 0.49%, equivalent to preventing 39 species each moving one Red List category closer to extinction between 1988 and 2008. For mammals, conservation action reduced the RLI decline from 0.94% to 0.8%, equivalent to preventing 29 species moving one category closer to extinction between 1996 and 2008.

These results grossly underestimate the impact of conservation, because they do not account for species that either (i) would have deteriorated further in the absence of conservation actions, or (ii) improved numerically, although not enough to change Red List status. As an example among the former, the black stilt (*Himantopus novaezelandiae*) would have gone extinct were it not for reintroduction and predator control efforts, and its Critically Endangered listing has thus remained unchanged (6). Among the latter, conservation efforts improved the total population numbers of 33 Critically Endangered birds during the period 1994 to 2004, but not sufficiently for any species to be moved to a lower category of threat (33). As many as 9% of mammals, birds, and amphibians classified as Threatened or Near Threatened have stable or increasing populations (15) largely due to conservation efforts, but it will take time for these successes to translate into improvements in status. Conservation efforts have also helped to avoid the deterioration in status of Least Concern species. Finally, conservation actions have benefited many other Threatened species besides birds, mammals, and amphibians, but this cannot yet be quantified through the RLI for groups that have been assessed only once [e.g., salmon shark (*Lamna ditropis*) numbers have improved as the result

of a 1992 U.N. moratorium on large-scale pelagic driftnet fisheries].

Confronting threats. Species recovery is complex and case-specific, but threat mitigation is always required. We investigated the main drivers of increased extinction risk by identifying, for each species that deteriorated in status, the primary threat responsible for that change. To understand which drivers of increased extinction risk are being mitigated most successfully, we identified, for each species that improved in status, the primary threat offset by successful conservation (table S6).

We found that for any single threat, regardless of the taxa involved, deteriorations outnumber improvements; conservation actions have not yet succeeded in offsetting any major driver of increased extinction risk (fig. S7). On a per-species basis, amphibians are in an especially dire situation, suffering the double jeopardy of exceptionally high levels of threat coupled with low levels of conservation effort. Still, there are conservation successes among birds and mammals, and here we investigate the degree to which particular threats have been addressed.

Conservation actions have been relatively successful at offsetting the threat of invasive alien species for birds and mammals: For every five species that deteriorated in status because of this threat, two improved through its mitigation. These successes have resulted from the implementation of targeted control or eradication programs [e.g., introduced cats have been eradicated from 37 islands since the mid-1980s (34)] coupled with reintroduction initiatives [e.g., the Seychelles magpie-robin (*Copsychus sechellarum*) population was 12 to 15 birds in 1965 but had increased to 150 birds by 2005 (fig. S8)]. Many of these improvements have occurred on small islands but also in Australia, owing in part to control of the red fox (*Vulpes vulpes*) (Fig. 4 and fig. S5B). However, among amphibians, only a single species—the Mallorcan midwife toad (*Alytes muletensis*)—improved in status as a result of mitigation of the threat posed by invasive alien species, compared with 208 species that deteriorated. This is because there is still a lack of understanding of the pathways by which chytridiomycosis is spread and may be controlled, and in situ conservation management options are only just beginning to be identified [e.g., (35)]. Meanwhile, the establishment of select, targeted captive populations with the goal of reintroducing species in the wild may offer valuable opportunities once impacts in their native habitat are brought under control [e.g., the Kihansi spray toad (*Nectophrynoides asperginis*), categorized as Extinct in the Wild because of drastic alteration of its spray zone habitat].

For mammals and birds, the threats leading to habitat loss have been less effectively addressed relative to that of invasive alien species: For every 10 species deteriorating as a

result of agricultural expansion, fewer than 1 improved because of mitigation of this threat. Protected areas are an essential tool to safeguard biodiversity from habitat loss, but the protected areas network remains incomplete and nonstrategic relative to Threatened species (17), and reserve management can be ineffective (36). Numerous Threatened species are restricted to single sites, many still unprotected (37), and these present key opportunities to slow rates of extinction. In the broader matrix of unprotected land, agri-environmental schemes could offer important biodiversity benefits, provided that management policies are sufficient to enhance populations of Threatened species (38).

Hunting has been relatively poorly addressed in mammals (62 deteriorations, 6 improvements) when compared with birds (31 deteriorations, 9 improvements). In birds, successes have resulted mainly from targeted protection [e.g., Lear's macaw (*Anodorhynchus leari*) changed from Critically Endangered to Endangered as a result of active protection of the Toca Velha/Serra Branca cliffs in Brazil], but also from enforcement of legislation (e.g., hunting bans) and harvest management measures. Many mammals subject to hunting occur at low densities, have large home ranges, and/or are large-bodied. Although active site-based protection has contributed to an improvement in the status of some of these species, site protection alone is often insufficient if not complemented by appropriate legislation, biological management, and effective enforcement (39). For example, a combination of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) and enactment of the Vicuña Convention, which prohibited domestic exploitation and mandated the establishment of protected areas, has helped to improve the status of the vicuña (*Vicuña vicugna*) from Near Threatened to Least Concern.

The threat of fisheries has been mitigated relatively more effectively for marine mammals (4 deteriorations, 2 improvements) than for birds (10 deteriorations, 0 improvements), reflecting both the time when drivers first emerged and the past influence of supranational conservation policy. Among historically exploited, long-lived mammals, for example, the humpback whale (*Megaptera novaeangliae*) has benefited from protection from commercial whaling (since 1955) and has improved from Vulnerable to Least Concern. Declines among slow-breeding seabirds (particularly albatrosses and petrels; fig. S9) are mainly a consequence of increasing incidental by-catch resulting from the growth of commercial fisheries, primarily those that use long-line and trawling methods. Legislative tools, such as the recently enacted multilateral Agreement on the Conservation of Albatrosses and Petrels (40), may yet deliver dividends by coordinating international action to reduce fisheries mortality of these highly migratory species.

Binding legislation and harvest management strategies also are urgently needed to address the disproportionate impact of fisheries on cartilaginous fishes (fig. S10).

We have no data on the relationship between expenditure on biodiversity and conservation success. A disproportionate percentage of annual conservation funding is spent in economically wealthy countries (41), where there are generally fewer Threatened species (Fig. 2 and fig. S4B) and the disparity between success and failure appears less evident (Fig. 4). Southeast Asia, by contrast, has the greatest imbalance between improving and deteriorating trends, emphasizing the need there for greater investment of resources and effort.

Conclusions. Our study confirms previous reports of continued biodiversity losses. We also find evidence of notable conservation successes illustrating that targeted, strategic conservation action can reduce the rate of loss relative to that anticipated without such efforts. Nonetheless, the current level of action is outweighed by the magnitude of threat, and conservation responses will need to be substantially scaled up to combat the extinction crisis. Even with recoveries, many species remain conservation-dependent, requiring sustained, long-term investment (42); for example, actions have been under way for 30 years for the golden lion tamarin (*Leontopithecus rosalia*), 70 years for the whooping crane (*Grus americana*), and 115 years for the white rhinoceros (*Ceratotherium simum*).

Halting biodiversity loss will require coordinated efforts to safeguard and effectively manage critical sites, complemented by broad-scale action to minimize further destruction, degradation, and fragmentation of habitats (37, 39) and to promote sustainable use of productive lands and waters in a way that is supportive to biodiversity. Effective implementation and enforcement of appropriate legislation could deliver quick successes; for example, by-catch mitigation measures, shark-finning bans, and meaningful catch limits have considerable potential to reduce declines in marine species (19). The 2010 biodiversity target may not have been met, but conservation efforts have not been a failure. The challenge is to remedy the current shortfall in conservation action to halt the attrition of global biodiversity.

References and Notes

1. S. H. M. Butchart *et al.*, *Science* **328**, 1164 (2010); 10.1126/science.1187512.
2. Secretariat of the Convention on Biological Diversity, *Global Biodiversity Outlook 3* (Convention on Biological Diversity, Montréal, 2010).
3. S. L. Pimm, G. J. Russell, J. L. Gittleman, T. M. Brooks, *Science* **269**, 347 (1995).
4. TEEB, *The Economics and Ecosystems of Biodiversity: An Interim Report* (European Communities, Cambridge, 2008).
5. UNEP, Report of the Sixth Meeting of the Conference of the Parties to the Convention on Biological Diversity (UNEP/CBD/COP/20/Part 2) Strategic Plan Decision VI/26

- in CBD (UNEP, Nairobi, 2002); www.cbd.int/doc/meetings/cop/cop-06/official/cop-06-20-en.pdf.
6. S. H. M. Butchart, A. J. Stattersfield, N. J. Collar, *Oryx* **40**, 266 (2006).
7. A. S. L. Rodrigues, *Science* **313**, 1051 (2006).
8. P. F. Donald *et al.*, *Science* **317**, 810 (2007).
9. T. M. Brooks, S. J. Wright, D. Sheil, *Conserv. Biol.* **23**, 1448 (2009).
10. P. J. Ferraro, S. K. Pattanayak, *PLoS Biol.* **4**, e105 (2006).
11. J. W. Terborgh, *Conserv. Biol.* **2**, 402 (1988).
12. B. Clucas, K. Mchugh, T. Caro, *Biodivers. Conserv.* **17**, 1517 (2008).
13. J. E. M. Baillie *et al.*, *Cons. Lett.* **1**, 18 (2008).
14. G. M. Mace *et al.*, *Conserv. Biol.* **22**, 1424 (2008).
15. IUCN Red List of Threatened Species. Version 2010.3 (IUCN, 2010; www.iucnredlist.org).
16. See supporting material on Science Online.
17. A. S. L. Rodrigues *et al.*, *Bioscience* **54**, 1092 (2004).
18. M. C. Hansen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 9439 (2008).
19. B. Worm *et al.*, *Science* **325**, 578 (2009).
20. S. H. M. Butchart *et al.*, *PLoS One* **2**, e140 (2007).
21. N. K. Dulvy, S. Jennings, S. I. Rogers, D. L. Maxwell, *Can. J. Fish. Aquat. Sci.* **63**, 1267 (2006).
22. B. Collen *et al.*, *Conserv. Biol.* **23**, 317 (2009).
23. N. S. Sodhi, L. P. Koh, B. W. Brook, P. K. Ng, *Trends Ecol. Evol.* **19**, 654 (2004).
24. S. M. Stuart *et al.*, *Science* **306**, 1783 (2004); 10.1126/science.1103538.
25. D. B. Wake, V. T. Vredenburg, *Proc. Natl. Acad. Sci. U.S.A.* **105** (suppl. 1), 11466 (2008).
26. H. R. Akçakaya, S. H. M. Butchart, G. M. Mace, S. N. Stuart, C. Hilton-Taylor, *Glob. Change Biol.* **12**, 2037 (2006).
27. B. W. Brook *et al.*, *Biol. Lett.* **5**, 723 (2009).
28. W. F. Laurance, D. C. Useche, *Conserv. Biol.* **23**, 1427 (2009).
29. K. E. Carpenter *et al.*, *Science* **321**, 560 (2008); 10.1126/science.1159196.
30. S. T. Turvey *et al.*, *Biol. Lett.* **3**, 537 (2007).
31. N. Sitas, J. E. M. Baillie, N. J. B. Isaac, *Anim. Conserv.* **12**, 231 (2009).
32. C. Gascon *et al.*, Eds., *Amphibian Conservation Action Plan* (IUCN/SSC Amphibian Specialist Group, Gland, Switzerland, 2007).
33. M. de L. Brooke *et al.*, *Conserv. Biol.* **22**, 417 (2008).
34. M. Nogales *et al.*, *Conserv. Biol.* **18**, 310 (2004).
35. R. N. Harris *et al.*, *ISME J.* **3**, 818 (2009).
36. L. M. Curran *et al.*, *Science* **303**, 1000 (2004).
37. T. H. Ricketts *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 18497 (2005).
38. D. Kleijn *et al.*, *Ecol. Lett.* **9**, 243 (2006).
39. C. Boyd *et al.*, *Cons. Lett.* **1**, 37 (2008).
40. J. Cooper *et al.*, *Mar. Ornithol.* **34**, 1 (2006).
41. A. N. James, K. J. Gaston, A. Balmford, *Nature* **401**, 323 (1999).
42. J. M. Scott, D. D. Goble, A. M. Haines, J. A. Wiens, M. C. Neel, *Cons. Lett.* **3**, 91 (2010).
43. We are indebted to the more than 3000 species experts who devoted their knowledge, intellect, and time to the compilation of vertebrate data on the IUCN Red List. Full acknowledgments are provided in the supporting online material.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1194442/DC1
Materials and Methods
Figs. S1 to S10
Tables S1 to S9
References
Acknowledgments

29 June 2010; accepted 11 October 2010
Published online 26 October 2010;
10.1126/science.1194442

Toroidal Dipolar Response in a Metamaterial

T. Kaelberer,¹ V. A. Fedotov,^{1*} N. Papasimakis,¹ D. P. Tsai,^{2,3} N. I. Zheludev^{1*}

Toroidal multipoles are fundamental electromagnetic excitations different from those associated with the familiar charge and magnetic multipoles. They have been held responsible for parity violation in nuclear and particle physics, but direct evidence of their existence in classical electrodynamics has remained elusive. We report on the observation of a resonant electromagnetic response in an artificially engineered medium, or metamaterial, that cannot be attributed to magnetic or charge multipoles and can only be explained by the existence of a toroidal dipole. Our direct experimental evidence of the toroidal response brings attention to the often ignored electromagnetic interactions involving toroidal multipoles, which could be present in naturally occurring systems, especially at the macromolecule level, where toroidal symmetry is ubiquitous.

An electric (or charge) dipole results from a separation of positive and negative charges, whereas a magnetic dipole is produced by the closed circulation of an electric current (Fig. 1, A and B). Mathematically they appear in the series expansion (known as the multipole expansion) of an electromagnetic potential generated by a distribution of charges and currents (*1, 2*). The toroidal dipole, an elusive counterpart of the charge and magnetic dipoles, is produced by currents flowing on the surface of a torus along its meridians (Fig. 1C). Toroidal dipoles were first considered by Zel'dovich in 1957 [who called them anapoles (*3*)] and have already been acknowledged in nuclear and particle physics [see (*4, 5*) and references within]. First-principles calculations have revealed the existence of toroidal dipoles for certain molecular structures (*6*) and ferroelectric systems (*7*).

Toroidal dipoles and higher toroidal multipoles are the subject of growing interest because of their unusual electromagnetic properties. In particular, it has been shown that the strength of their interaction with electric and magnetic fields depends not on the strength of the fields, but rather on their time derivatives (*8*). It has also been suggested that nonstationary charge-current configurations involving toroidal multipoles could produce oscillating and propagating vector potential in the absence of electromagnetic fields (*9, 10*). Media containing molecules with elements of toroidal symmetry could rotate the polarization of light (*11*) or could exhibit a negative index of refraction (*12*). Afanasiev (*13*) claimed that interactions between electrical currents producing toroidal multipoles would violate Newton's Third Law, which requires that the mutual forces between two interacting bodies are equal, opposite, and colinear.

Furthermore, a material with domains of toroidal polarization is expected to have different optical properties along opposite directions (*14*).

Toroidal multipoles are not a part of the standard multipole expansion (*15*). The electromagnetic fields generated by an arbitrary system of nonstationary charges and currents are usually presented as a sum of contributions from two families of conventional elementary radiation sources: the magnetic and electric dynamic multipoles (*1, 2*). In a spherical coordinate system, magnetic multipoles are defined by transversal components of oscillating current density (currents flowing on the surface of a sphere), whereas electric multipoles are attributed to oscillating charge density (charge multipoles). Radiating fields, however, also contain contributions from oscillating radial components of the current density. These radial components of the current density lead to the existence of the independent family of dynamic toroidal multipoles that are different from the magnetic and charge multipoles (*16–18*).

The most primitive member of the toroidal multipole family is the toroidal dipole **T** (*15*). It is created by poloidal currents (currents flowing on the surface of a torus along its meridians), but can be equivalently represented by a set of magnetic dipoles **m** arranged head-to-tail along a loop (Fig. 1C) (*15*). The toroidal dipole points outward along the symmetry axis of the torus. Toroidal coils and molecular structures alike could also display toroidal dipole moments (*6, 17*). The electromagnetic manifestations of the toroidal dipole, however, are usually masked by much stronger effects due to charge and magnetic mul-

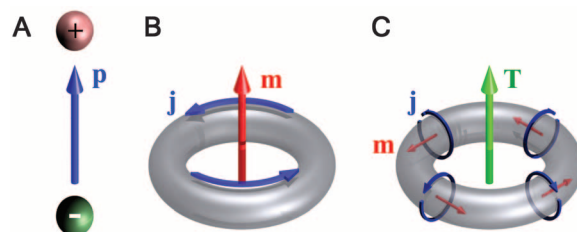
tipoles, making observations of the toroidal response extremely challenging (*14, 19, 20*).

We demonstrate a classical system in which the electromagnetic response is directly related to the resonant excitation of the toroidal dipole. The resonant toroidal response has been observed in a "metamaterial slab," a two-dimensional array of artificially engineered electromagnetic scatterers of toroidal symmetry. Metamaterials enable us to access novel and exotic electromagnetic phenomena not found in nature (such as negative refraction and cloaking) by controlling the symmetry and character of the response through artificial structuring on a subwavelength scale. To emphasize the toroidal dipolar response, we have designed a metamolecule, the elementary building block of our metamaterial, where both the electric and magnetic dipole moments induced by an incident electromagnetic wave (as well as higher multipoles) are substantially suppressed while the toroidal response is spectrally isolated and resonantly enhanced to a detectable level.

We depart from the obvious toroidal solenoid-like wire configuration, as it would also support a strong magnetic dipole moment because of the helical nature of its windings (*13*). Our toroidal metamolecule is composed of four rectangular, electrically disconnected metallic wire loops ($a \times h$) embedded into a low-loss dielectric slab of overall size $d \times d \times s$. The loops are located in two mutually orthogonal planes and separated by a distance r (Fig. 2A). The intersection of the planes gives the axis of the toroidal structure (parallel to the z axis). All wire loops have identical splits g , which are located either on the top or bottom sides of the slab such that the entire metamolecule has an inversion center C located on the axis of the structure (Fig. 2A). The metamolecule is placed in a rectangular unit cell $d \times d \times s$, which is periodically translated along y and z axes to form a metamaterial slab.

The structure of our metamolecule ensures some distinct electromagnetic properties. Apart from supporting a magnetic dipole mode, now routinely observed in other metamaterials (*21*), it supports a dominant mode of a toroidal dipole nature. Excitations of both modes are manifested as resonant features **I** and **II** in the metamaterial's transmission and reflection spectra, which were simulated and measured for an electromagnetic wave polarized along the z axis (Fig. 3, A and B). The resonances result from the circular currents induced by the incident wave in all four loops of

Fig. 1. (A) A pair of charges of opposite signs creates an electric (charge) dipole **p**. (B) A current **j** flowing along a loop produces a magnetic dipole **m**. (C) Currents flowing on a surface of a torus along its meridians (poloidal currents) generate a toroidal dipole **T**. The toroidal dipole can also be represented as a closed loop of magnetic dipoles arranged head-to-tail.



¹Optoelectronics Research Centre and Centre for Photonic Metamaterials, University of Southampton, Southampton SO17 1BJ, UK. ²Department of Physics, National Taiwan University, Taipei 10617, Taiwan. ³Instrument Technology Research Center, National Applied Research Laboratories, Hsinchu 300, Taiwan.

*To whom correspondence should be addressed. E-mail: vaf@orc.soton.ac.uk (V.A.F.); niz@orc.soton.ac.uk (N.I.Z.); Web: www.nanophotonics.org.uk

the metamolecule. For z -polarization, these currents cannot be excited by the electric component of the wave, as it is orthogonal to the split of the loops. Correspondingly, the z -component of the electric dipole moment of the system P_z is suppressed for both resonances. The metamolecule interacts with the magnetic part of the wave, which drives circular currents in accordance with Faraday's law of induction. When passing through the metamaterial slab, the wave reaches the front and rear pairs of the loops of the metamolecule with a phase delay. Thus, the magnetic field at the loops can be decomposed into parallel (in-phase) and antiparallel (antiphase) components. At resonance *I* the metamolecule interacts with the in-phase components of the magnetic field. The induced magnetic dipoles of all four loops $\mathbf{m}$ point in the same direction, producing a nonzero component of the metamolecule's net magnetic dipole moment M_y parallel to the driving magnetic field (Fig. 2B), which reveals the magnetic origin of the resonance. By contrast, at resonance *II* the metamolecule couples to the antiphase components of the magnetic field and the individual magnetic moments induced in the front and rear pairs of the loops are directed oppositely, forming a head-to-tail configuration (Fig. 2C). Such a head-to-tail configuration of the individual magnetic moments $\mathbf{m}$ has a nonzero component of the induced toroidal dipole mo-

ment T_z oriented along the axis of the metamolecule (z axis), whereas its net magnetic dipole and quadrupole moments are negligible.

The qualitative arguments produced above are fully supported by our calculations performed using a full three-dimensional Maxwell equations solver based on the finite element method. We modeled the interaction of the metamaterial array with a linearly polarized wave in the spectral range from 14.5 to 17.0 GHz. Calculated densities of the induced electrical currents were used to compute scattered powers of the resulting conventional magnetic and charge multipoles, as well as the toroidal dipole of the metamolecule (Fig. 3C) (22).

The strongest contribution to the metamaterial response at resonance *I* is provided by the y -component of the magnetic dipole moment M_y . Here it radiates more strongly than the electric quadrupole moment by a factor of ~ 5 , and more strongly than the dominant radiating component of the electric dipole P_z and the magnetic quadrupole by several orders of magnitude. Its resonant excitation manifests itself as a peak in reflection and deep in transmission at ~ 16.1 GHz, with a quality factor Q of ~ 80 (Fig. 3, A and B). The nature of the resonance is illustrated in Fig. 3D, where we plot the distribution of the magnetic field lines in the vicinity of the metamolecule. It shows that the field lines are split in two

bundles as they thread the wire loops of the metamolecule, and rejoin immediately outside the structure running along the magnetic field of the incident wave. Such a field line configuration indicates strong coupling of the magnetic dipole mode to the free space, thus explaining the moderate value of the Q factor.

Resonance *II* is located at ~ 15.4 GHz and is also seen as a peak in reflection and dip in transmission, but with the Q factor reaching 240 (Fig. 3, A and B). Magnetic dipole and electric quadrupole excitations of the metamolecule are not resonant at this frequency. Moreover, here the scattering efficiency of electric and magnetic multipoles, which is directly related to the metamaterial's reflectivity, is low. Therefore, the conventional multipole excitations cannot be responsible for the resonance feature at 15.4 GHz. This indicates that the resonance may be due to a toroidal dipole, which scatters more strongly than any of the conventional multipoles by almost two orders of magnitude (Fig. 3C). The toroidal nature of this excitation is illustrated in Fig. 3E, where the calculated lines of the local magnetic field are seen to form closed loops that are largely confined within the metamolecule (as in a true toroidal coil), giving rise to the z -component of the toroidal dipole moment T_z . The higher Q factor of this mode (in comparison with the magnetic di-

Fig. 2. Electromagnetic metamaterial supporting toroidal dipolar excitation. (A) Unit cell of the metamaterial, containing four split wire loops embedded into a dielectric slab. (B and C) Two distinctively different modes of excitation corresponding to magnetic (*I*) and toroidal (*II*) dipole resonances, respectively. (D) Close-up photograph of the wire structure of the toroidal metamolecule. (E) Assembled metamaterial slab, 8 mm $\times$ 176 mm $\times$ 165 mm (green solder resist was removed before the measurements).

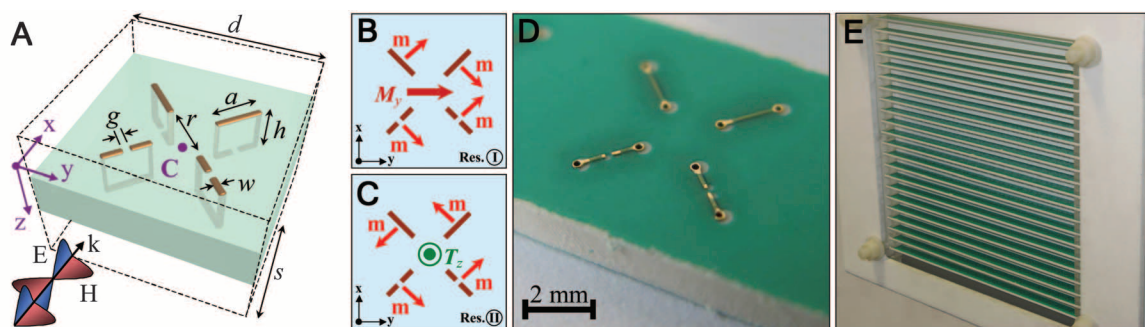
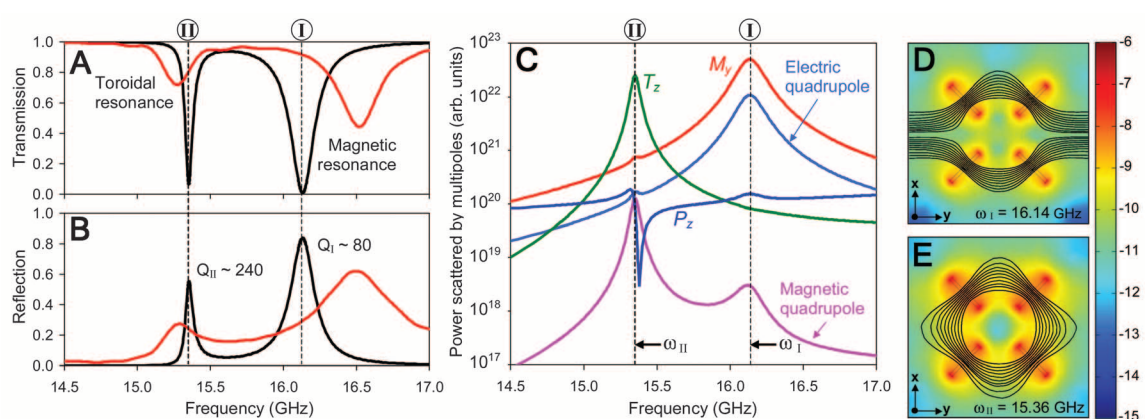


Fig. 3. Resonant toroidal response of the metamaterial. (A and B) Calculated (black lines) and measured (red lines) transmission and reflection spectra of the metamaterial. Positions of the calculated magnetic and toroidal dipole resonances, *I* and *II*, are marked by vertical dashed lines. (C) Dispersion of scattered power for various multipole moments induced in the metamolecule that contribute to the reflection and transmission spectra of the metamaterial slab. (D and E) Distributions of magnetic field lines for correspondingly magnetic (*I*) and toroidal (*II*) dipole resonances plotted over the color maps of the magnetic energy density (logarithmic scale).



pole mode I) is the direct consequence of its strong confinement and weak free-space coupling. Note that at the toroidal resonance, the electrical dipole excitation has a strong net x -component P_x , which results from the presence of the splits in the loops of the metamolecule. However, as P_x oscillates along the wave propagation direction, it does not contribute to the metamaterial's reflection and transmission.

We observed the toroidal dipole resonance experimentally in a metamaterial slab formed by a 22×22 array of toroidal metamolecules (Fig. 2D). The rows of the array were manufactured from metalized microwave laminate strips by high-resolution printed board technology; these rows were than stacked at a regular interval with the axes of the metamolecules aligned in the plane of the array (Fig. 2E). The transmission and reflection spectra of the slab were measured in an anechoic chamber by means of a vector network analyzer and linearly polarized horn antennas (22). The experimental data (Fig. 3, A and B) show good agreement with the simulated spectra. The somewhat lower Q factors of the measured resonances are attributed to the manufacturing tolerance and limited size of the metamaterial array, as well as to some divergence of the wave front of the incident microwave beam. The slight mismatch between the calculated and measured values of the resonance frequencies (0.7% and 2% for the corresponding toroidal and magnetic resonances, respectively) is due to the limitations of the fabrication process (22), which did not allow perfect replication of the design features,

such as the profile of the wires forming the loops; this could affect the wires' inductance and intramolecular interactions.

Toroidal multipoles are routinely neglected in the constitutive relations, boundary conditions, electromagnetic forces, and calculation of momentum loss and radiation intensity of charge-current configurations (5, 23–25). Our results provide compelling evidence of a resonant response that can only be attributed to a toroidal dipole excitation of the metamaterial structure in the microwave part of the spectrum. Downsizing the structure should allow observation of a plasmonic toroidal mode at optical frequencies in a single submicrometer metallic toroid, as well as in arrays of such toroids. Molecular systems such as fullerene rings are also predicted to support toroidal moments (6), thus urging the development of a quantum mechanical description of the light-matter interaction with toroidal molecular structures. Moreover, our results call for the development of a deeper understanding of the quantum mechanism of intramolecular interactions involving toroidal molecular structures, which are widespread in nature.

References and Notes

1. J. D. Jackson, *Classical Electrodynamics* (Wiley, New York, ed. 3, 1999), chap. 16.
2. L. D. Landau, E. M. Lifschitz, *Course of Theoretical Physics* (Pergamon, New York, ed. 4, 1993), vol. 2.
3. Ia. B. Zel'dovich, *Sov. Phys. JETP* **6**, 1184 (1958).
4. W. C. Haxton, *Science* **275**, 1753 (1997).
5. E. E. Radescu, G. Vaman, *Phys. Rev. E* **65**, 046609 (2002).

6. A. Ceulemans, L. F. Chibotaru, P. Fowler, *Phys. Rev. Lett.* **80**, 1861 (1998).
7. I. I. Naumov, L. Bellaiche, H. Fu, *Nature* **432**, 737 (2004).
8. V. M. Dubovik, L. A. Tosunyan, V. V. Tugushev, *Sov. Phys. JETP* **63**, 344 (1986).
9. G. N. Afanasiev, V. M. Dubovik, *Phys. Part. Nucl.* **29**, 366 (1998).
10. A. D. Boardman, K. Marinov, N. Zheludev, V. A. Fedotov, *Phys. Rev. E* **72**, 036603 (2005).
11. N. Papasimakis, V. A. Fedotov, K. Marinov, N. I. Zheludev, *Phys. Rev. Lett.* **103**, 093901 (2009).
12. K. Marinov, A. D. Boardman, V. A. Fedotov, N. Zheludev, *N. J. Phys.* **9**, 324 (2007).
13. G. N. Afanasiev, *J. Phys. D* **34**, 539 (2001).
14. K. Sawada, N. Nagaosa, *Phys. Rev. Lett.* **95**, 237402 (2005).
15. V. M. Dubovik, V. V. Tugushev, *Phys. Rep.* **187**, 145 (1990).
16. V. M. Dubovik, A. A. Cheshkov, *Sov. J. Part. Nucl.* **5**, 318 (1974).
17. G. N. Afanasiev, V. M. Dubovik, *J. Phys. A* **25**, 4869 (1992).
18. A. Góngora T., E. Ley-Koo, *Rev. Mex. Fis.* **E 52**, 188 (2006).
19. V. A. Fedotov, K. Marinov, A. D. Boardman, N. I. Zheludev, *N. J. Phys.* **9**, 95 (2007).
20. S. Alborghetti *et al.*, *N. J. Phys.* **10**, 063019 (2008).
21. D. R. Smith, J. B. Pendry, M. C. K. Wiltshire, *Science* **305**, 788 (2004).
22. See supporting material on Science Online.
23. E. V. Tkalya, *Phys. Rev. A* **65**, 022504 (2002).
24. V. M. Dubovik, M. A. Martsenyuk, B. Saha, *Phys. Rev. E* **61**, 7087 (2000).
25. H. Schmid, *Ferroelectrics* **252**, 41 (2001).
26. Supported by the UK Engineering and Physical Sciences Research Council, the Royal Society, and the Leverhulme Trust.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1197172/DC1
Materials and Methods

Fig. S1

References

31 August 2010; accepted 19 October 2010

Published online 4 November 2010;

10.1126/science.1197172

Evidence of Supersolidity in Rotating Solid Helium

H. Choi,¹ D. Takahashi,² K. Kono,² E. Kim^{1*}

Supersolidity, the appearance of zero-viscosity flow in solids, was first indicated in helium-4 torsional oscillator (TO) experiments. In this apparatus, the irrotationality of the superfluid component causes it to decouple from the underlying normal solid, leading to a reduction in the resonant period of the TO. However, the resonant period may be altered for reasons other than supersolidity, such as the temperature dependence of the elastic modulus of solid helium. Superimposing rotation onto oscillatory measurements may distinguish between supersolidity and classical effects. We performed such simultaneous measurements of the TO and the shear modulus, and observed substantial change in the resonant period with rotational speed where the modulus remained unchanged. This contrasting behavior suggests that the decrease in the TO period is a result of supersolidity.

Because of its irrotational nature, a superfluid decouples from its container when subject to rotation, leading to a reduction of rotational inertia at the superfluid transition. The missing rotational inertia is referred to as non-

classical rotation inertia (NCRI) (I). In addition to the fluid ^4He , the reduction of the resonant period of a torsional oscillator (TO) was also found in solid ^4He and was interpreted as a manifestation of a paradoxical state of matter: supersolidity (2, 3).

Since the initial discovery, a number of puzzles have arisen, such as the lack of a critical exponent at the transition, extremely low critical velocity (2–4), dc flow that depends on experimental conditions (5–11), and lack of the second or fourth sound (12, 13). Without direct evidence of phase

coherence in solid ^4He , a different interpretation of TO experiments is possible (14–16). In a perfectly rigid solid, all parts of the solid oscillate perfectly in phase with the torsional cell. The resonant frequency ω_0 of an ideal TO is directly related to I , the moment of inertia of the torsion bob, and is described by $\omega_0 = \sqrt{K/I}$, where K is the spring constant of the torsion rod. For a viscoelastic solid, on the other hand, parts of the solid could lag behind the cell and build stress within the solid. As a consequence, the resonant period shift may not be affected by the change in rotational inertia only, but may also reflect a change in the temperature-dependent viscoelasticity of the solid. In oscillatory experiments, the resonant period can therefore change without invoking supersolidity.

This scenario is supported by a marked resemblance between the TO and shear modulus measurements (17). However, estimates of the viscoelastic effects based on the magnitude of the measured shear modulus change do not fully account for the size of the period change observed in TO measurements. This inconsistency has partially inspired alternative compound models such as the superglass (18).

A more recent phenomenological model that includes a contribution from the additional strain originating from sliding dislocations (19) was also

¹Department of Physics and Center for Supersolid and Quantum Matter Research, KAIST, Daejeon 305-701, Republic of Korea. ²Low Temperature Physics Laboratory, RIKEN, Wako-shi, Saitama 351-0198, Japan.

*To whom correspondence should be addressed. E-mail: eunseong@kaist.edu

pole mode I) is the direct consequence of its strong confinement and weak free-space coupling. Note that at the toroidal resonance, the electrical dipole excitation has a strong net x -component P_x , which results from the presence of the splits in the loops of the metamolecule. However, as P_x oscillates along the wave propagation direction, it does not contribute to the metamaterial's reflection and transmission.

We observed the toroidal dipole resonance experimentally in a metamaterial slab formed by a 22×22 array of toroidal metamolecules (Fig. 2D). The rows of the array were manufactured from metalized microwave laminate strips by high-resolution printed board technology; these rows were than stacked at a regular interval with the axes of the metamolecules aligned in the plane of the array (Fig. 2E). The transmission and reflection spectra of the slab were measured in an anechoic chamber by means of a vector network analyzer and linearly polarized horn antennas (22). The experimental data (Fig. 3, A and B) show good agreement with the simulated spectra. The somewhat lower Q factors of the measured resonances are attributed to the manufacturing tolerance and limited size of the metamaterial array, as well as to some divergence of the wave front of the incident microwave beam. The slight mismatch between the calculated and measured values of the resonance frequencies (0.7% and 2% for the corresponding toroidal and magnetic resonances, respectively) is due to the limitations of the fabrication process (22), which did not allow perfect replication of the design features,

such as the profile of the wires forming the loops; this could affect the wires' inductance and intramolecular interactions.

Toroidal multipoles are routinely neglected in the constitutive relations, boundary conditions, electromagnetic forces, and calculation of momentum loss and radiation intensity of charge-current configurations (5, 23–25). Our results provide compelling evidence of a resonant response that can only be attributed to a toroidal dipole excitation of the metamaterial structure in the microwave part of the spectrum. Downsizing the structure should allow observation of a plasmonic toroidal mode at optical frequencies in a single submicrometer metallic toroid, as well as in arrays of such toroids. Molecular systems such as fullerene rings are also predicted to support toroidal moments (6), thus urging the development of a quantum mechanical description of the light-matter interaction with toroidal molecular structures. Moreover, our results call for the development of a deeper understanding of the quantum mechanism of intramolecular interactions involving toroidal molecular structures, which are widespread in nature.

References and Notes

1. J. D. Jackson, *Classical Electrodynamics* (Wiley, New York, ed. 3, 1999), chap. 16.
2. L. D. Landau, E. M. Lifschitz, *Course of Theoretical Physics* (Pergamon, New York, ed. 4, 1993), vol. 2.
3. Ia. B. Zel'dovich, *Sov. Phys. JETP* **6**, 1184 (1958).
4. W. C. Haxton, *Science* **275**, 1753 (1997).
5. E. E. Radescu, G. Vaman, *Phys. Rev. E* **65**, 046609 (2002).

6. A. Ceulemans, L. F. Chibotaru, P. Fowler, *Phys. Rev. Lett.* **80**, 1861 (1998).
7. I. I. Naumov, L. Bellaiche, H. Fu, *Nature* **432**, 737 (2004).
8. V. M. Dubovik, L. A. Tosunyan, V. V. Tugushev, *Sov. Phys. JETP* **63**, 344 (1986).
9. G. N. Afanasiev, V. M. Dubovik, *Phys. Part. Nucl.* **29**, 366 (1998).
10. A. D. Boardman, K. Marinov, N. Zheludev, V. A. Fedotov, *Phys. Rev. E* **72**, 036603 (2005).
11. N. Papasimakis, V. A. Fedotov, K. Marinov, N. I. Zheludev, *Phys. Rev. Lett.* **103**, 093901 (2009).
12. K. Marinov, A. D. Boardman, V. A. Fedotov, N. Zheludev, *N. J. Phys.* **9**, 324 (2007).
13. G. N. Afanasiev, *J. Phys. D* **34**, 539 (2001).
14. K. Sawada, N. Nagaosa, *Phys. Rev. Lett.* **95**, 237402 (2005).
15. V. M. Dubovik, V. V. Tugushev, *Phys. Rep.* **187**, 145 (1990).
16. V. M. Dubovik, A. A. Cheshkov, *Sov. J. Part. Nucl.* **5**, 318 (1974).
17. G. N. Afanasiev, V. M. Dubovik, *J. Phys. A* **25**, 4869 (1992).
18. A. Góngora T., E. Ley-Koo, *Rev. Mex. Fis.* **E 52**, 188 (2006).
19. V. A. Fedotov, K. Marinov, A. D. Boardman, N. I. Zheludev, *N. J. Phys.* **9**, 95 (2007).
20. S. Alborghetti *et al.*, *N. J. Phys.* **10**, 063019 (2008).
21. D. R. Smith, J. B. Pendry, M. C. K. Wiltshire, *Science* **305**, 788 (2004).
22. See supporting material on Science Online.
23. E. V. Tkalya, *Phys. Rev. A* **65**, 022504 (2002).
24. V. M. Dubovik, M. A. Martsenyuk, B. Saha, *Phys. Rev. E* **61**, 7087 (2000).
25. H. Schmid, *Ferroelectrics* **252**, 41 (2001).
26. Supported by the UK Engineering and Physical Sciences Research Council, the Royal Society, and the Leverhulme Trust.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1197172/DC1
Materials and Methods

Fig. S1

References

31 August 2010; accepted 19 October 2010

Published online 4 November 2010;

10.1126/science.1197172

Evidence of Supersolidity in Rotating Solid Helium

H. Choi,¹ D. Takahashi,² K. Kono,² E. Kim^{1*}

Supersolidity, the appearance of zero-viscosity flow in solids, was first indicated in helium-4 torsional oscillator (TO) experiments. In this apparatus, the irrotationality of the superfluid component causes it to decouple from the underlying normal solid, leading to a reduction in the resonant period of the TO. However, the resonant period may be altered for reasons other than supersolidity, such as the temperature dependence of the elastic modulus of solid helium. Superimposing rotation onto oscillatory measurements may distinguish between supersolidity and classical effects. We performed such simultaneous measurements of the TO and the shear modulus, and observed substantial change in the resonant period with rotational speed where the modulus remained unchanged. This contrasting behavior suggests that the decrease in the TO period is a result of supersolidity.

Because of its irrotational nature, a superfluid decouples from its container when subject to rotation, leading to a reduction of rotational inertia at the superfluid transition. The missing rotational inertia is referred to as non-

classical rotation inertia (NCRI) (I). In addition to the fluid ^4He , the reduction of the resonant period of a torsional oscillator (TO) was also found in solid ^4He and was interpreted as a manifestation of a paradoxical state of matter: supersolidity (2, 3).

Since the initial discovery, a number of puzzles have arisen, such as the lack of a critical exponent at the transition, extremely low critical velocity (2–4), dc flow that depends on experimental conditions (5–11), and lack of the second or fourth sound (12, 13). Without direct evidence of phase

coherence in solid ^4He , a different interpretation of TO experiments is possible (14–16). In a perfectly rigid solid, all parts of the solid oscillate perfectly in phase with the torsional cell. The resonant frequency ω_0 of an ideal TO is directly related to I , the moment of inertia of the torsion bob, and is described by $\omega_0 = \sqrt{K/I}$, where K is the spring constant of the torsion rod. For a viscoelastic solid, on the other hand, parts of the solid could lag behind the cell and build stress within the solid. As a consequence, the resonant period shift may not be affected by the change in rotational inertia only, but may also reflect a change in the temperature-dependent viscoelasticity of the solid. In oscillatory experiments, the resonant period can therefore change without invoking supersolidity.

This scenario is supported by a marked resemblance between the TO and shear modulus measurements (17). However, estimates of the viscoelastic effects based on the magnitude of the measured shear modulus change do not fully account for the size of the period change observed in TO measurements. This inconsistency has partially inspired alternative compound models such as the superglass (18).

A more recent phenomenological model that includes a contribution from the additional strain originating from sliding dislocations (19) was also

¹Department of Physics and Center for Supersolid and Quantum Matter Research, KAIST, Daejeon 305-701, Republic of Korea. ²Low Temperature Physics Laboratory, RIKEN, Wako-shi, Saitama 351-0198, Japan.

*To whom correspondence should be addressed. E-mail: eunseong@kaist.edu

introduced to overcome this discrepancy. The results of a TO experiment comparing a sample before and after plastic deformation are consistent with this non-supersolid model (20).

The TO and shear modulus measurements are both ac-driven experimental techniques; this makes it difficult to distinguish between supersolid and classical effects. We investigated an alternative approach that involves superimposition of a continuous (dc) rotation onto the ac oscillation of the TO. Quantum mechanical processes would be subject to change under dc rotation, whereas sim-

ple material properties such as the shear modulus would not.

This approach was previously taken by Gumann *et al.*, who cooled a TO down to ~ 50 mK with rotational speeds as high as 1.25 rad/s (21). Gumann *et al.* observed increased dissipation without any marked change in the period under increasing rotational speeds. This was interpreted as the existence of a three-dimensionally coherent supersolid state. However, the results were open to other non-supersolid explanations. For example, mechanical vibrations associated with the

rotation would influence the dissipation more than they would the period. Because the elastic modulus could not be monitored in their experiment, it was not clear whether the additional dissipation could have come from unexpected changes in the elastic modulus.

To eliminate these shortcomings, we improved the experimental conditions by achieving a lower base temperature (15 mK) and a higher rotational speed (5 rad/s), and investigated simultaneously the elastic modulus under rotation to distinguish the classical effects from supersolidity.

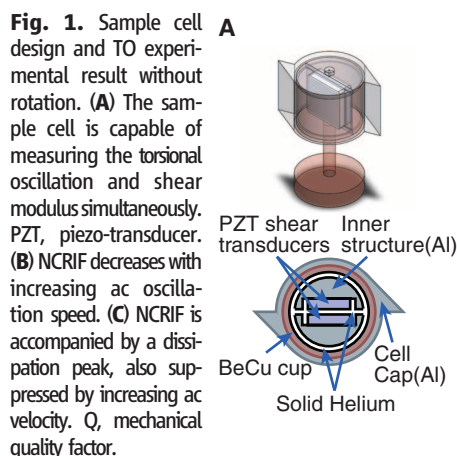
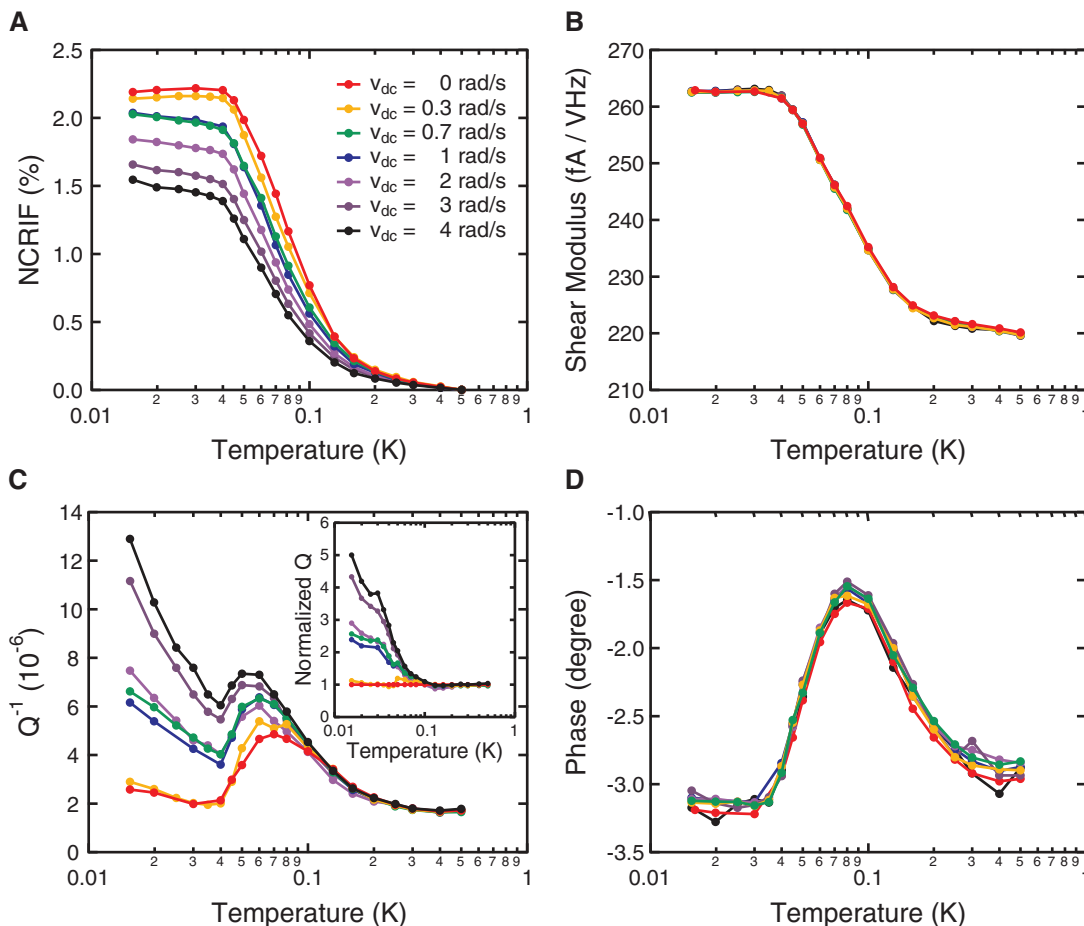


Fig. 2. Experimental results under dc rotation. **(A)** NCRIF is suppressed with growing dc rotation speed. **(B)** Shear modulus is not affected by dc rotation. **(C)** At low temperatures there is additional dissipation, which scales with the rotation speed. This low-temperature dissipation is separated out in the inset by normalizing to zero rotation dissipation (red circles). **(D)** Phase shift in the piezo-transducer indicates that the dissipation in the solid is related to the shear modulus measurement. Rotation has no effect on this dissipation.



We grew solid helium with the blocked-capillary method so that at the end of the crystallization, the cell contained a sample at 40 bar. In addition to an annular channel, the cell has a center channel along the diameter, on either side of which are two piezo-transducers for measuring shear modulus simultaneously. The outer diameter of the annular channel is 16 mm, and the width of both the annular channel and the center channel is 0.4 mm (Fig. 1A). The mechanical quality factor of the empty cell measured at 1 K is 10^6 .

We first measured the temperature-dependent period and amplitude of the TO under various ac oscillation speeds. The resonant period change and dissipation at different ac velocities are shown in Fig. 1, B and C, respectively. Typical TO measurement results were reproduced (2, 3). The maximum NCRIF fraction (NCRIF) was measured to be 2.2%, and the ac critical velocity was found to be $10 \mu\text{m/s}$. Beyond $10 \mu\text{m/s}$, NCRIF became smaller with increasing velocity, and this was accompanied by a decreasing and narrowing dissipation peak. Both NCRIF and the dissipation peak ultimately vanished at around 0.6 mm/s .

We then fixed the ac oscillation speed at $6 \mu\text{m/s}$ and performed the same measurements under different dc rotation speeds (Fig. 2). The piezo-transducer used for the shear modulus measurement was driven at 1 kHz with a 5-mV drive. To avoid confusion with the ac oscillation speed, when discussing dc rotation we use the angular velocity in units of rad/s. Multiplying by a conversion factor of 8 mm/rad gives us the dc rotation speed at the rim of the torsion cell. The maximum rotation speed achieved was 5 rad/s (40 mm/s). It is clear from Fig. 3A that increasing the dc rotation speed causes suppression in NCRIF by as much as 40% at 5 rad/s .

All non-supersolid models rely on the temperature-dependent viscoelasticity of the solid as the reason for the reduction of the TO period. A back-action term that is a function of classical material properties, such as the shear modulus, is necessary in the equation of motion to account for the effects of viscoelasticity; dc rotation may affect these classical properties, if a sufficiently large stress

can be built within a solid by either the centrifugal force or vibrations caused by the rotation.

To test this hypothesis, we measured the shear modulus simultaneously with the resonant period and found that it did not change under rotation (Fig. 2B). The effect of rotation should be the same for both the resonant period and the shear modulus if the two are affected by a common microscopic mechanism, although the degree of susceptibility to rotation may vary depending on the sample quality and geometry. The shift in the shear modulus caused by rotation was less than the experimental resolution: 0.3% of the total modulus change. The unaffected modulus is not surprising because the maximum pressure felt by the solid helium in the annular channel as a result of the centrifugal force is only about 0.01 Pa even at 5 rad/s , too small to cause softening. Changes in the TO resonant period therefore cannot be accounted for by the shear modulus change alone.

As seen by the shear modulus anomaly persisting even when NCRIF is suppressed under dc rotation, the increase in shear modulus with cooling is more robust than that of NCRIF. Thus, any connection between the two phenomena must be indirect. One likely scenario is that the phase coherence required for supersolidity can easily be destroyed by the motion of unpinned dislocations, and the shear modulus change is a necessary condition for the emergence of NCRIF.

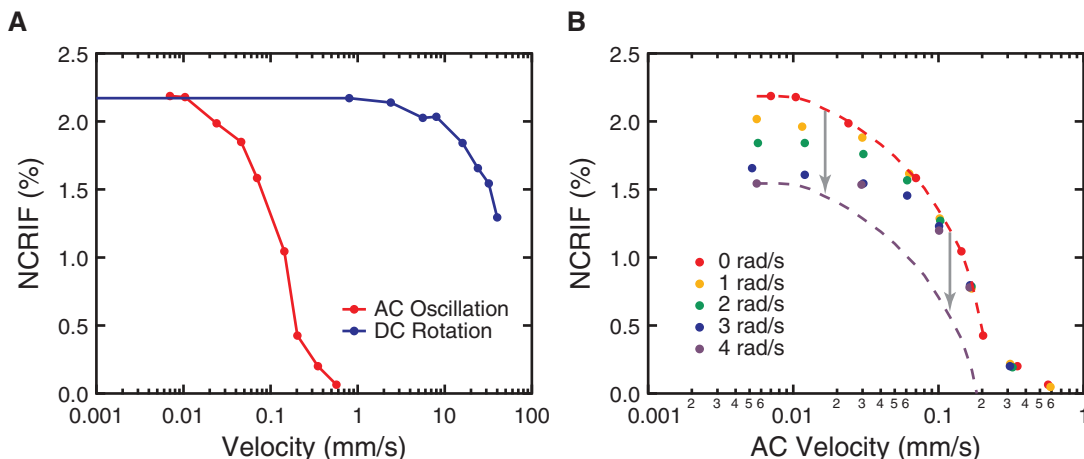
Another pronounced effect of dc rotation is that the suppression of NCRIF by rotation is not accompanied by a gradual disappearance of the dissipation peak as seen in the ac oscillation studies. Instead, extra dissipation arises at temperatures below 100 mK (Fig. 2C). This is contradictory to the predictions of non-supersolid models in which the period change and the dissipation peak are the real and imaginary parts of the rotational susceptibility, respectively. According to non-supersolid models, the suppression (or enhancement) of the period change should be accompanied by a suppression (or enhancement) of the dissipation peak, regardless of the suppression mechanism. However, this concurrent suppression appeared only when the period drop was suppressed by increasing the

ac velocity of the TO. In contrast, our measurements under dc rotation show the suppression of the period change with an unusual enhancement of the dissipation, also indicating that the period drop in a TO is not a manifestation of solid stiffening.

The suppression in the NCRIF became noticeable when the rotation speed exceeded $\sim 1 \text{ mm/s}$, which is much greater than the ac critical velocity of $10 \mu\text{m/s}$ (Fig. 3A). One reasonable way to explain this difference is that dc rotation results in a different mechanism for the suppression of NCRIF than does ac oscillation. This is supported further by the observation that the dissipation behaves differently under the influence of dc rotation and large ac oscillation. Combined with the large dissipation we see, it seems that high-speed dc rotation creates vortices, in possible analogy to the similar findings on two-dimensional superfluid films under rotation (22). The ac critical speed of $10 \mu\text{m/s}$ is then far too small for vortex injection to take place. Therefore, the critical value that was obtained by the past ac TO experiments (23) may not be analogous to the critical velocity of more conventional superfluids, the point of vortex injection. The true critical velocity of the ^4He supersolid can be seen only through dc rotation and is on the order of $\sim 1 \text{ mm/s}$. This is much closer to the values reported for other superfluids.

Given the oscillatory nature of the TO technique, the origin of the suppression of NCRIF and the behavior of the dissipation peak with increasing oscillation speed is still unclear. The dynamic response of the vortices or dislocations in solid ^4He could be responsible for the suppression (14, 17). To study the nature of the ac oscillation-induced suppression carefully, we superposed high-speed ac oscillations upon various dc rotation speeds. When dc rotation and sufficiently large ac oscillation are superposed, a crossover from a dc rotation-dominated NCRIF suppression to an ac oscillation-dominated NCRIF suppression appears (Fig. 3B). The presence of this crossover implies that dc rotation and ac oscillation influence a common physical entity that is responsible for the suppression of NCRIF. Otherwise, the two processes would suppress NCRIF independently, and

Fig. 3. Critical velocity of the supersolid. All the data shown were obtained at the base temperature of 15 mK . **(A)** NCRIF suppression is plotted against ac oscillation speed and dc rotation speed. The dc critical velocity appears to be two orders of magnitude larger than the ac critical velocity. **(B)** Measurements of ac critical velocity under various dc rotation speeds. The dashed red curve is the guide for the eye. The gray arrows and the dashed purple curve indicate the hypothetical suppression of NCRIF at 4 rad/s if one assumes that the ac and dc mechanisms for the suppression have independent origins.



the dc rotation simply would have provided extra suppression by a uniform amount on top of the ac oscillation suppression, as illustrated by the purple dashed curve in Fig. 3B.

As the suppressed NCRIF and the enhanced dissipation under dc rotation are thought to be coming from vortex injection, this shared common origin could be vortices. If so, the competition between vortex injection and vortex motion is one possible explanation for this crossover. A large ac oscillation amplitude causes unpinning of the vortices; the oscillatory motion of these unpinned vortices overwhelms the effect of injected quantized vortices above a certain crossover ac oscillation speed. The gradual enhancement of the crossover speed is also seen with increasing dc speed and can be attributed to the larger number of unpinned vortices that are required to observe the crossover. We note that this oscillation amplitude is still very small, orders of magnitude smaller than the critical strain for the softening of solid helium.

The appearance of extra dissipation only at low temperatures is, however, puzzling, as vortex

pinning is thought to be stronger at these temperatures (24). The lack of similarity to better-understood superfluids and the lack of a quantitative theory for supersolid vortices makes it difficult to explain this peculiar feature.

References and Notes

- A. J. Leggett, *Phys. Rev. Lett.* **25**, 1543 (1970).
- E. Kim, M. H. W. Chan, *Nature* **427**, 225 (2004).
- E. Kim, M. H. W. Chan, *Science* **305**, 1941 (2004).
- N. Prokof'ev, *Adv. Phys.* **56**, 381 (2007).
- J. Day, T. Herman, J. Beamish, *Phys. Rev. Lett.* **95**, 035301 (2005).
- J. Day, J. Beamish, *Phys. Rev. Lett.* **96**, 105304 (2006).
- A. S. C. Rittner, W. Choi, E. J. Mueller, J. D. Reppy, *Phys. Rev. B* **80**, 224516 (2009).
- M. W. Ray, R. B. Hallock, *Phys. Rev. Lett.* **100**, 235301 (2008).
- M. W. Ray, R. B. Hallock, *Phys. Rev. B* **79**, 224302 (2009).
- M. W. Ray, R. B. Hallock, *Phys. Rev. B* **82**, 012502 (2010).
- M. W. Ray, R. B. Hallock, *Phys. Rev. Lett.* **105**, 145301 (2010).
- Y. Aoki, H. Kojima, X. Lin, *Low Temp. Phys.* **34**, 329 (2008).
- S. Kwon, N. Mulders, E. Kim, *J. Low Temp. Phys.* **158**, 590 (2009).
- P. W. Anderson, *Nat. Phys.* **3**, 160 (2007).
- Z. Nussinov, A. V. Balatsky, M. J. Graf, S. A. Trugman, *Phys. Rev. B* **76**, 014530 (2007).
- C.-D. Yoo, A. T. Dorsey, *Phys. Rev. B* **79**, 100504 (2009).
- J. Day, J. Beamish, *Nature* **450**, 853 (2007).
- B. Hunt *et al.*, *Science* **324**, 632 (2009).
- I. Iwasa, *Phys. Rev. B* **81**, 104527 (2010).
- J. D. Reppy, *Phys. Rev. Lett.* **104**, 255301 (2010).
- P. Gumann, N. Shimizu, A. Penzev, Y. Yasuta, M. Kubota, *J. Phys. Conf. Ser.* **150**, 032026 (2009).
- T. Obata, M. Fukuda, N. Mikhin, J. D. Reppy, M. Kubota, *J. Low Temp. Phys.* **134**, 559 (2004).
- E. Kim, M. H. W. Chan, *Phys. Rev. Lett.* **97**, 115302 (2006).
- H. Choi, S. Kwon, D. Y. Kim, E. Kim, *Nat. Phys.* **6**, 424 (2010).
- Supported by the National Research Foundation of Korea through Creative Research Initiatives and the Japan Society for the Promotion of Science through a Grant-in-Aid for Scientific Research. We thank members of the Center for Supersolid and Quantum Matter Research, in particular D. Y. Kim and S. Kwon, for helpful discussions.

12 August 2010; accepted 8 November 2010

Published online 18 November 2010;

10.1126/science.1196409

In Situ Observation of the Electrochemical Lithiation of a Single SnO₂ Nanowire Electrode

Jian Yu Huang,^{1*} Li Zhong,² Chong Min Wang,^{3*} John P. Sullivan,^{1*} Wu Xu,⁴ Li Qiang Zhang,² Scott X. Mao,^{2*} Nicholas S. Hudak,¹ Xiao Hua Liu,¹ Arunkumar Subramanian,¹ Hongyou Fan,⁵ Liang Qi,^{6,7} Akihiro Kushima,⁷ Ju Li^{6,7*}

We report the creation of a nanoscale electrochemical device inside a transmission electron microscope—consisting of a single tin dioxide (SnO₂) nanowire anode, an ionic liquid electrolyte, and a bulk lithium cobalt dioxide (LiCoO₂) cathode—and the in situ observation of the lithiation of the SnO₂ nanowire during electrochemical charging. Upon charging, a reaction front propagated progressively along the nanowire, causing the nanowire to swell, elongate, and spiral. The reaction front is a “Medusa zone” containing a high density of mobile dislocations, which are continuously nucleated and absorbed at the moving front. This dislocation cloud indicates large in-plane misfit stresses and is a structural precursor to electrochemically driven solid-state amorphization. Because lithiation-induced volume expansion, plasticity, and pulverization of electrode materials are the major mechanical effects that plague the performance and lifetime of high-capacity anodes in lithium-ion batteries, our observations provide important mechanistic insight for the design of advanced batteries.

Lithiation and delithiation of the electrode materials in lithium-ion batteries (LIBs) induce large strains in the host material, leading to plasticity and fracture. Lithiation is also often accompanied by phase transformations, such as electrochemically driven solid-state amorphization (ESA) (1). These electrochemical reaction-induced microstructural events limit the energy capacity and cycle lifetime of LIBs (2–6). It was recently reported that lithium-ion anode materials composed of nanowires (7–12) can offer improved performance and lifetime relative to those of micrometer-scale or larger materials. The improvements are often attributed to the nanowire's unique geometry and enhanced accommodation of the transformation strains that occur during cycling

(9, 10, 13, 14). However, the detailed mechanisms of strain-induced plasticity and strain accommodation in nanowires during electrochemical charging are largely unknown.

We have successfully constructed a nanoscale electrochemical device consisting of a single SnO₂ nanowire as an anode, an ionic liquid-based electrolyte (ILE), and a cathode of LiCoO₂ particles inside a high-resolution transmission electron microscope (HRTEM) (Fig. 1A) to enable direct real-time visualization of electrochemical reaction-induced microstructural changes. As shown in Fig. 1B, the initial SnO₂ nanowire was straight with a smooth surface morphology. After contact with the ILE, the ILE wicked up the nanowire, forming a meniscus (Fig. 1C). Potential was then

applied to the SnO₂ nanowire at −3.5 V with respect to the LiCoO₂ counterelectrode. This initiated an electrochemical reaction at the point of contact between the SnO₂ nanowire and the ILE where reduction of the SnO₂ was observed. This solid-state reaction front propagated along the longitudinal direction of the nanowire away from the electrolyte (Fig. 1, D to S, and movie S1). As the reaction front propagated, the diameter and length of the nanowire increased, and the TEM image contrast changed from typical crystalline diffraction contrast to a gray, mostly featureless contrast typical of amorphous materials (Fig. 2 and Fig. 3). At 625 s (Fig. 1, I and P to S, and movie S1), the nanowire began to flex rapidly, which resulted in the formation of a bend and the start of a coil of a spiral. After 1860 s of charging, the initially straight nanowire (Fig. 1B) exhibited a twisted and meandering morphology (Fig. 1O), indicative of extensive plastic deformation and microstructural changes. It took about half an hour to charge a nanowire with initial length of 16 μm and diameter of 188 nm. After charging, this nanowire had elongated ~60%, the diameter expanded ~45%, and the total volume expanded about 240%.

¹Center for Integrated Nanotechnologies, Sandia National Laboratories, Albuquerque, NM 87185, USA. ²Department of Mechanical Engineering and Materials Science, University of Pittsburgh, Pittsburgh, PA 15261, USA. ³Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA 99354, USA. ⁴Energy and Environment Directorate, Pacific Northwest National Laboratory, Richland, WA 99354, USA. ⁵Advanced Materials Lab, Sandia National Laboratories, Albuquerque, NM 87106, USA. ⁶State Key Laboratory for Mechanical Behavior of Materials and Frontier Institute of Science and Technology, Xi'an Jiaotong University, Xi'an 710049, China. ⁷Department of Materials Science and Engineering, University of Pennsylvania, Philadelphia, PA 19104, USA.

*To whom correspondence should be addressed. E-mail: jhuang@sandia.gov (J.Y.H.); chongmin.wang@pnl.gov (C.M.W.); jpsulli@sandia.gov (J.P.S.); sxm2@pitt.edu (S.X.M.); liju@seas.upenn.edu (J.L.)

the dc rotation simply would have provided extra suppression by a uniform amount on top of the ac oscillation suppression, as illustrated by the purple dashed curve in Fig. 3B.

As the suppressed NCRIF and the enhanced dissipation under dc rotation are thought to be coming from vortex injection, this shared common origin could be vortices. If so, the competition between vortex injection and vortex motion is one possible explanation for this crossover. A large ac oscillation amplitude causes unpinning of the vortices; the oscillatory motion of these unpinned vortices overwhelms the effect of injected quantized vortices above a certain crossover ac oscillation speed. The gradual enhancement of the crossover speed is also seen with increasing dc speed and can be attributed to the larger number of unpinned vortices that are required to observe the crossover. We note that this oscillation amplitude is still very small, orders of magnitude smaller than the critical strain for the softening of solid helium.

The appearance of extra dissipation only at low temperatures is, however, puzzling, as vortex

pinning is thought to be stronger at these temperatures (24). The lack of similarity to better-understood superfluids and the lack of a quantitative theory for supersolid vortices makes it difficult to explain this peculiar feature.

References and Notes

- A. J. Leggett, *Phys. Rev. Lett.* **25**, 1543 (1970).
- E. Kim, M. H. W. Chan, *Nature* **427**, 225 (2004).
- E. Kim, M. H. W. Chan, *Science* **305**, 1941 (2004).
- N. Prokof'ev, *Adv. Phys.* **56**, 381 (2007).
- J. Day, T. Herman, J. Beamish, *Phys. Rev. Lett.* **95**, 035301 (2005).
- J. Day, J. Beamish, *Phys. Rev. Lett.* **96**, 105304 (2006).
- A. S. C. Rittner, W. Choi, E. J. Mueller, J. D. Reppy, *Phys. Rev. B* **80**, 224516 (2009).
- M. W. Ray, R. B. Hallock, *Phys. Rev. Lett.* **100**, 235301 (2008).
- M. W. Ray, R. B. Hallock, *Phys. Rev. B* **79**, 224302 (2009).
- M. W. Ray, R. B. Hallock, *Phys. Rev. B* **82**, 012502 (2010).
- M. W. Ray, R. B. Hallock, *Phys. Rev. Lett.* **105**, 145301 (2010).
- Y. Aoki, H. Kojima, X. Lin, *Low Temp. Phys.* **34**, 329 (2008).
- S. Kwon, N. Mulders, E. Kim, *J. Low Temp. Phys.* **158**, 590 (2009).
- P. W. Anderson, *Nat. Phys.* **3**, 160 (2007).
- Z. Nussinov, A. V. Balatsky, M. J. Graf, S. A. Trugman, *Phys. Rev. B* **76**, 014530 (2007).
- C.-D. Yoo, A. T. Dorsey, *Phys. Rev. B* **79**, 100504 (2009).
- J. Day, J. Beamish, *Nature* **450**, 853 (2007).
- B. Hunt *et al.*, *Science* **324**, 632 (2009).
- I. Iwasa, *Phys. Rev. B* **81**, 104527 (2010).
- J. D. Reppy, *Phys. Rev. Lett.* **104**, 255301 (2010).
- P. Gumann, N. Shimizu, A. Penzev, Y. Yasuta, M. Kubota, *J. Phys. Conf. Ser.* **150**, 032026 (2009).
- T. Obata, M. Fukuda, N. Mikhin, J. D. Reppy, M. Kubota, *J. Low Temp. Phys.* **134**, 559 (2004).
- E. Kim, M. H. W. Chan, *Phys. Rev. Lett.* **97**, 115302 (2006).
- H. Choi, S. Kwon, D. Y. Kim, E. Kim, *Nat. Phys.* **6**, 424 (2010).
- Supported by the National Research Foundation of Korea through Creative Research Initiatives and the Japan Society for the Promotion of Science through a Grant-in-Aid for Scientific Research. We thank members of the Center for Supersolid and Quantum Matter Research, in particular D. Y. Kim and S. Kwon, for helpful discussions.

12 August 2010; accepted 8 November 2010

Published online 18 November 2010;

10.1126/science.1196409

In Situ Observation of the Electrochemical Lithiation of a Single SnO₂ Nanowire Electrode

Jian Yu Huang,^{1*} Li Zhong,² Chong Min Wang,^{3*} John P. Sullivan,^{1*} Wu Xu,⁴ Li Qiang Zhang,² Scott X. Mao,^{2*} Nicholas S. Hudak,¹ Xiao Hua Liu,¹ Arunkumar Subramanian,¹ Hongyou Fan,⁵ Liang Qi,^{6,7} Akihiro Kushima,⁷ Ju Li^{6,7*}

We report the creation of a nanoscale electrochemical device inside a transmission electron microscope—consisting of a single tin dioxide (SnO₂) nanowire anode, an ionic liquid electrolyte, and a bulk lithium cobalt dioxide (LiCoO₂) cathode—and the in situ observation of the lithiation of the SnO₂ nanowire during electrochemical charging. Upon charging, a reaction front propagated progressively along the nanowire, causing the nanowire to swell, elongate, and spiral. The reaction front is a “Medusa zone” containing a high density of mobile dislocations, which are continuously nucleated and absorbed at the moving front. This dislocation cloud indicates large in-plane misfit stresses and is a structural precursor to electrochemically driven solid-state amorphization. Because lithiation-induced volume expansion, plasticity, and pulverization of electrode materials are the major mechanical effects that plague the performance and lifetime of high-capacity anodes in lithium-ion batteries, our observations provide important mechanistic insight for the design of advanced batteries.

Lithiation and delithiation of the electrode materials in lithium-ion batteries (LIBs) induce large strains in the host material, leading to plasticity and fracture. Lithiation is also often accompanied by phase transformations, such as electrochemically driven solid-state amorphization (ESA) (1). These electrochemical reaction-induced microstructural events limit the energy capacity and cycle lifetime of LIBs (2–6). It was recently reported that lithium-ion anode materials composed of nanowires (7–12) can offer improved performance and lifetime relative to those of micrometer-scale or larger materials. The improvements are often attributed to the nanowire's unique geometry and enhanced accommodation of the transformation strains that occur during cycling

(9, 10, 13, 14). However, the detailed mechanisms of strain-induced plasticity and strain accommodation in nanowires during electrochemical charging are largely unknown.

We have successfully constructed a nanoscale electrochemical device consisting of a single SnO₂ nanowire as an anode, an ionic liquid-based electrolyte (ILE), and a cathode of LiCoO₂ particles inside a high-resolution transmission electron microscope (HRTEM) (Fig. 1A) to enable direct real-time visualization of electrochemical reaction-induced microstructural changes. As shown in Fig. 1B, the initial SnO₂ nanowire was straight with a smooth surface morphology. After contact with the ILE, the ILE wicked up the nanowire, forming a meniscus (Fig. 1C). Potential was then

applied to the SnO₂ nanowire at −3.5 V with respect to the LiCoO₂ counterelectrode. This initiated an electrochemical reaction at the point of contact between the SnO₂ nanowire and the ILE where reduction of the SnO₂ was observed. This solid-state reaction front propagated along the longitudinal direction of the nanowire away from the electrolyte (Fig. 1, D to S, and movie S1). As the reaction front propagated, the diameter and length of the nanowire increased, and the TEM image contrast changed from typical crystalline diffraction contrast to a gray, mostly featureless contrast typical of amorphous materials (Fig. 2 and Fig. 3). At 625 s (Fig. 1, I and P to S, and movie S1), the nanowire began to flex rapidly, which resulted in the formation of a bend and the start of a coil of a spiral. After 1860 s of charging, the initially straight nanowire (Fig. 1B) exhibited a twisted and meandering morphology (Fig. 1O), indicative of extensive plastic deformation and microstructural changes. It took about half an hour to charge a nanowire with initial length of 16 μm and diameter of 188 nm. After charging, this nanowire had elongated ~60%, the diameter expanded ~45%, and the total volume expanded about 240%.

¹Center for Integrated Nanotechnologies, Sandia National Laboratories, Albuquerque, NM 87185, USA. ²Department of Mechanical Engineering and Materials Science, University of Pittsburgh, Pittsburgh, PA 15261, USA. ³Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA 99354, USA. ⁴Energy and Environment Directorate, Pacific Northwest National Laboratory, Richland, WA 99354, USA. ⁵Advanced Materials Lab, Sandia National Laboratories, Albuquerque, NM 87106, USA. ⁶State Key Laboratory for Mechanical Behavior of Materials and Frontier Institute of Science and Technology, Xi'an Jiaotong University, Xi'an 710049, China. ⁷Department of Materials Science and Engineering, University of Pennsylvania, Philadelphia, PA 19104, USA.

*To whom correspondence should be addressed. E-mail: jhuang@sandia.gov (J.Y.H.); chongmin.wang@pnl.gov (C.M.W.); jpsulli@sandia.gov (J.P.S.); sxm2@pitt.edu (S.X.M.); liju@seas.upenn.edu (J.L.)

The large shape change of the nanowire during charging was a general feature of all the nanowires that were investigated. In fig. S1, A to K, we show the structural changes of another SnO_2 nanowire before and after charging, in this case polarized to -4 V with respect to the LiCoO_2 cathode. It took about 80 min to charge this nanowire with an initial length of $14\ \mu\text{m}$ and diameter of $107\ \text{nm}$. After charging, the initially straight nanowire became highly distorted, with a total elongation of 90%, a diameter expansion of 35%, and a total volume expansion of 250%. Figure S2 shows the charging dynamics of a third nanowire. Large shape changes were observed again.

Figure 2 shows a more detailed structure and phase characterization of the nanowire before and after charging. Close inspection of the reaction front (Fig. 2A) revealed the presence of a region of a high density of dislocations separating

the nonreacted and reacted segments of the nanowire. Before reaction, the nanowire was straight and monocrystalline, as revealed by the electron diffraction pattern (EDP) (Fig. 2B). Immediately after charging, the nanowire showed a dark gray contrast (Fig. 2A), and the EDP of most areas showed amorphous haloes (Fig. 2D). After prolonged charging, the nanowire comprised small nanocrystals dispersed in an amorphous matrix (Fig. 2F), and the EDP showed diffraction rings superimposed on diffuse amorphous haloes (Fig. 2E); the diffraction rings could be indexed as hexagonal Li_xSn (orange indices in Fig. 2E) and tetragonal Sn (black indices in Fig. 2E). The EDP from the reaction front (Fig. 2C) showed diffraction spots superimposed on a diffuse scattering background. These diffraction spots are similar to that of the nonreacted nanowire, except that the zone axis of the former is slightly tilted

with respect to the latter. Electron energy loss spectroscopy (EELS) indicated that, after reaction, the nanowire contained metallic Sn, Li, and Li_2O (Fig. 2, G and H). EELS from a charged segment of the nanowire, such as that shown in Fig. 2A, showed the presence of Li (Fig. 2G, red line profile). The Li-K edge is similar to that of Li_2O rather than metallic Li (15), indicating that the amorphous phase is Li_2O . Occasionally, an EDP revealed the presence of nanocrystalline Li_2O , which was found in the nanowire after charging (fig. S3). These results revealed that the nanowire after charging consists of nanocrystalline Li_xSn and Sn particles dispersed in an amorphous Li_2O matrix.

In total, these measurements reveal that when a SnO_2 nanowire was polarized at a sufficiently negative potential with respect to LiCoO_2 , the SnO_2 was initially reduced to nanocrystalline Sn

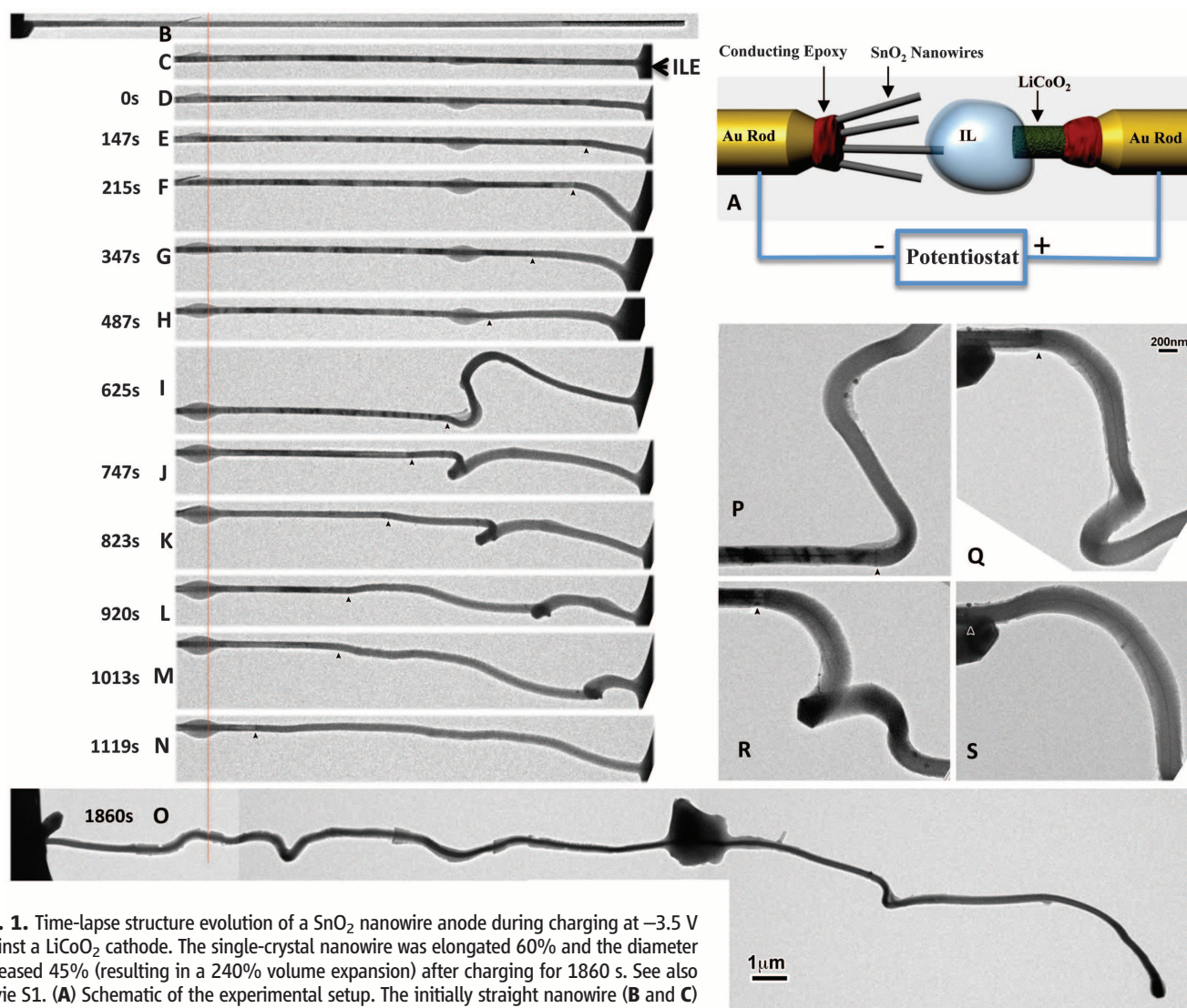


Fig. 1. Time-lapse structure evolution of a SnO_2 nanowire anode during charging at -3.5 V against a LiCoO_2 cathode. The single-crystal nanowire was elongated 60% and the diameter increased 45% (resulting in a 240% volume expansion) after charging for 1860 s. See also movie S1. (A) Schematic of the experimental setup. The initially straight nanowire (B and C) became significantly twisted and bent after charging (D to S). The chemical reaction front progressed along the nanowire's longitudinal direction, with the front clearly visible, as pointed out by arrowheads in (E) to (S). The red line in (B) to (O) marks a reference point to track the change of the nanowire length. (P) to (S) are sequential high-magnification images showing the progressive migration of the reaction front, swelling, and the twisted morphology of the nanowire after the reaction front passed by. The big dark particle in the middle of (O) is an island of gelled ILE. Because of the long cumulative electron beam exposure time during the recording of TEM images, the ILE front became gelled (with high viscosity) at this spot.

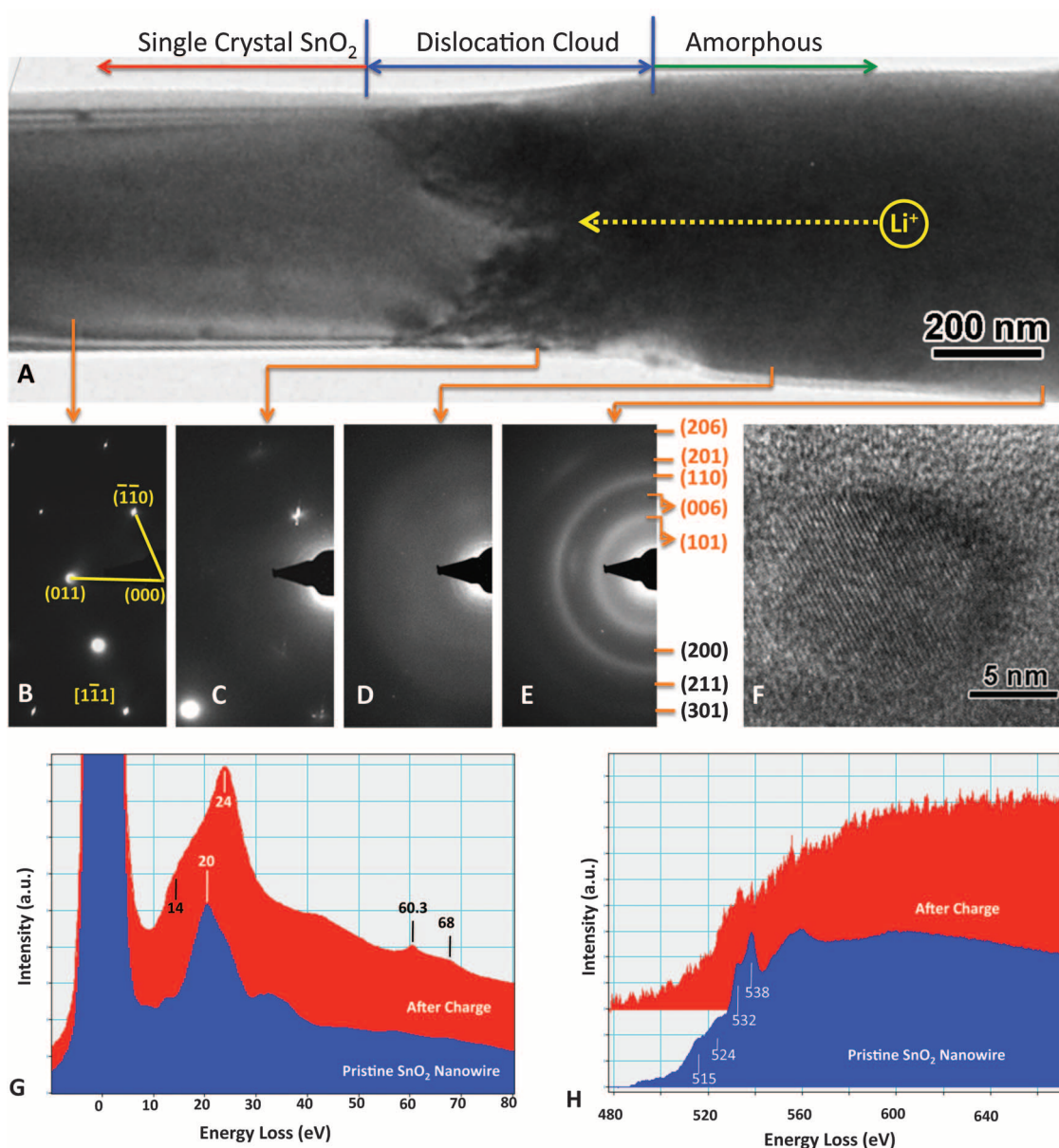
and amorphous Li_2O , which suggests the following reduction reaction: $4\text{Li}^+ + \text{SnO}_2 + 4\text{e}^- \rightarrow 2\text{Li}_2\text{O} + \text{Sn}$. This is the “forming stage” to produce a Sn-containing anode. After this initial phase transformation, the operation of the Sn- LiCoO_2 battery is based on a reversible reaction, such as $\text{Sn} + x\text{Li}^+ + x\text{e}^- \leftrightarrow \text{Li}_x\text{Sn}$ ($0 \leq x \leq 4.4$) (16). While reduction was occurring at the SnO_2 nanowire anode, at the LiCoO_2 cathode Co was being oxidized from Co^{3+} to Co^{4+} , and Li^+ ions were expelled; that is, $\text{LiCoO}_2 \rightarrow \text{Li}_{1-\delta}\text{CoO}_2 + \delta\text{Li}^+ + \delta\text{e}^-$.

In contrast to bulk SnO_2 , which is a brittle ceramic, nanowire SnO_2 showed large plasticity (as evidenced by the high dislocation density at the reaction front), and we did not observe fracture or cracking despite the high strain at the reacting interface. Details of the generation and migration

of the dislocations near the reacting interface are shown in Fig. 3 and movies S2 to S5. In SnO_2 , the width of this region of high dislocation density was $\sim 10^2$ nm, and is named the “Medusa zone” because of the appearance of dislocations “snaking” away from the interface. It may occur in certain electrochemical solid-state reactions, and its existence indicates very high stresses at the reaction front; the high stress drives dislocation nucleation and motion. Previously, ex situ TEM studies showed that a high density of dislocations may exist in LiCoO_2 cathodes in LIBs as a result of electrochemical cycling (17). However, it is far from clear when and how these dislocations are generated and how they evolve during cycling. Our in situ movies show that the dislocations were continuously nucleated in the crystal regions

and then moved away from the highly stressed region. They were also pursued from behind and absorbed by the moving amorphous-crystalline interface (ACI) (18), thereby maintaining a steady state in the total dislocation cloud, which migrated in an approximate chevron shape along the nanowire. One type of dislocation in SnO_2 is determined to be of $[01\bar{1}](100)$ slip character (fig. S4). We have performed ab initio density functional theory (DFT) calculations and found the ideal shear strength of SnO_2 to be ~ 10 GPa. Because a very high density of dislocations was seen to be nucleated readily and continuously at the interface even as the old nucleation zone was being demolished by the advancing reaction front (which would imply the removal of the original Frank-Read dislocation sources), we postulate that

Fig. 2. Structural and phase characterization of another SnO_2 nanowire anode during charging at -3.5 V against the LiCoO_2 cathode. (A) TEM micrograph of the nanowire containing a reaction front (“dislocation cloud”) separating the reacted (“amorphous”) and nonreacted (“single-crystal SnO_2 ”) sections. (B to E) EDPs from the different sections of the nanowire. The pristine nanowire was single crystalline and the corresponding EDP (B) can be indexed as the $[1\bar{1}1]$ zone axis of rutile SnO_2 . The EDP from the dislocation zone (C) shows a spot pattern superimposed on a diffuse scattering background. The EDP from an area immediately after the reaction front (D) shows an amorphous halo. The EDP from an area far away from the reaction front (E) shows diffraction rings superimposed on a diffuse amorphous halo. The diffraction rings can be indexed as tetragonal Sn (black indices) and a Li_xSn compound such as hexagonal $\text{Li}_{13}\text{Sn}_5$ (orange indices). (F) A HRTEM image from a charged nanowire showing Sn nanoparticles dispersed in an amorphous matrix. (G to H) Low-loss and core-loss EELS from a large area of the nanowire after reaction (red line profile) and a pristine nanowire (blue line profile). The pristine SnO_2 shows two characteristic core-loss peaks at 515 and 524 eV, corresponding to the $\text{Sn-M}_{4,5}$ edge riding on a delayed edge. The peaks at 532 and 538 eV arise from the O-K edge. Note that Li is present in the charged nanowire (G). The plasmon loss peaks at 20 eV, 24 eV, and 14 eV are in excellent agreement with SnO_2 , Li_2O , and pure Sn, respectively.



a stress close to the ideal strength (14) should exist in the Medusa zone. Such a large stress would be expected at the reaction interface, as the reacted side of the interface exhibits a 45% increase in radial expansion relative to the unreacted side. This would generate a large tensile stress near the ACI that leads to spontaneous dislocation nucleation on the unreacted side, and a large compressive stress on the reacted amorphous side. Plasticity is expected to also occur on the amorphous side (18, 19), despite the lack of dislocations.

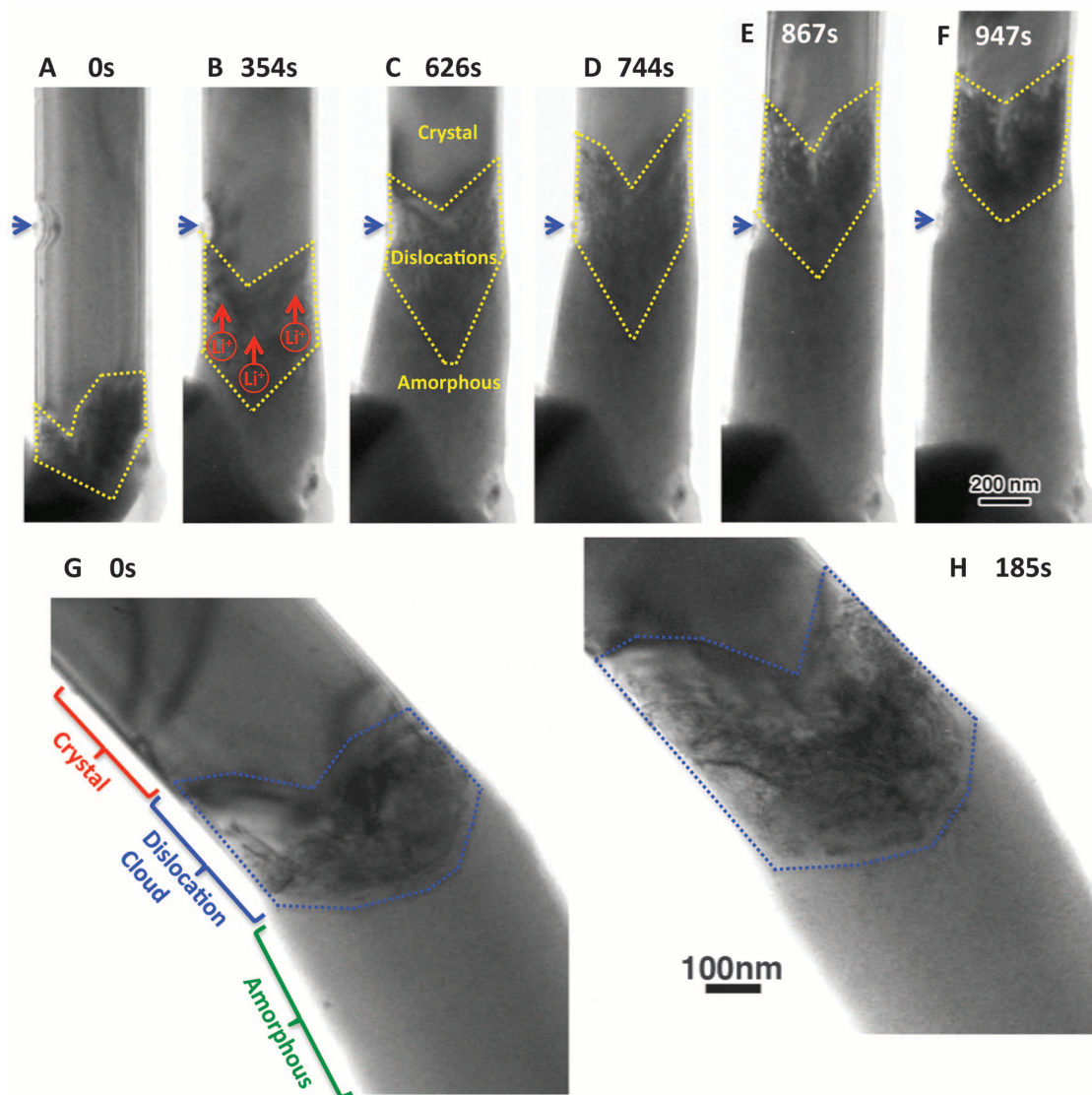
We note two important consequences of the observed dislocation structure and dynamics at the reacting interface. First, the dislocation cores may be highly effective Li transport channels (20) and may facilitate Li ion insertion into the crystalline interior, effectively increasing the reaction kinetics. Second, the amorphous phase we observed in situ did not form via the melt-quench mechanism, but via a direct crystal-to-glass transition (i.e., ESA) (1). Solid-state amorphization (21–23) has often been associated with mechanical alloying of bulk materials (e.g., ball-milling)

(24–26). Here it is observed in the context of an electrochemical reaction with large stress and apparent dislocation plasticity at the reaction front. Fortunately, electron transparency allows us to capture the dynamical process of ESA with TEM. Our observations suggest that stress-driven dislocation plasticity may be a precursor to some solid-state amorphizations (23–26). The dislocation density we observed in the Medusa zone was exceptionally high, on the order of $10^{17}/\text{m}^2$, which is about two orders of magnitude larger than that in heavily work-hardened face-centered cubic metals (27). Such a high dislocation density was caused by the exceptionally high stress driven by the electrochemical reaction. The dislocation cloud disturbs the structural order of the crystal and drives it far from equilibrium. This can provide the necessary energy and kinetic pathway toward complete amorphization.

In addition to the interesting precipitate/dislocation microstructures, we observed very unusual gross morphological changes of the nanowire. Elastic energy strongly influences the shape

of phase transformation products (28), and the nanowire geometry provides an elasticity boundary condition very different from that of 3D bulk materials. For the SnO_2 nanowire polarized at -4 V versus LiCoO_2 (fig. S1), we observed a very large anisotropy in the transformation strain, namely $\sim 90\%$ elongation in the $\langle 011 \rangle$ axial direction compared to $\sim 35\%$ expansion in the transverse directions; the total volume expanded by $\sim 250\%$. Our DFT calculation gives a net volume expansion that matches very well with the experimental result (table S1), assuming $x = 3$ in the charging reaction $(4+x)\text{Li}^+ + \text{SnO}_2 + (4+x)\text{e}^- \rightarrow 2\text{Li}_2\text{O} + \text{Li}_x\text{Sn}$. But the calculated transformation strain anisotropy, based on uniform electrochemical Li^+ insertions alone, is completely different; that is, the largest expansion should occur along $\langle 001 \rangle$ instead of $\langle 011 \rangle$. We interpret this contrast as due to the buckling instability of the nanowire (movie S1); the wire is elastically very compliant in the axial direction and therefore prefers to accommodate the volume expansion in the axial direction. In the transverse directions, because of geometric con-

Fig. 3. TEM images revealed a high density of dislocations emerging from the reaction front (marked by chevron-shaped dotted lines). As the dislocation front propagated, the crystalline contrast changed to gray amorphous contrast instantaneously, and the nanowire diameter increased immediately. See also movies S2 to S5. (A to F) and (G and H) Two sets of time-lapsed TEM images showing the high density of dislocations that appeared at the reaction front and the migration of the reaction front.



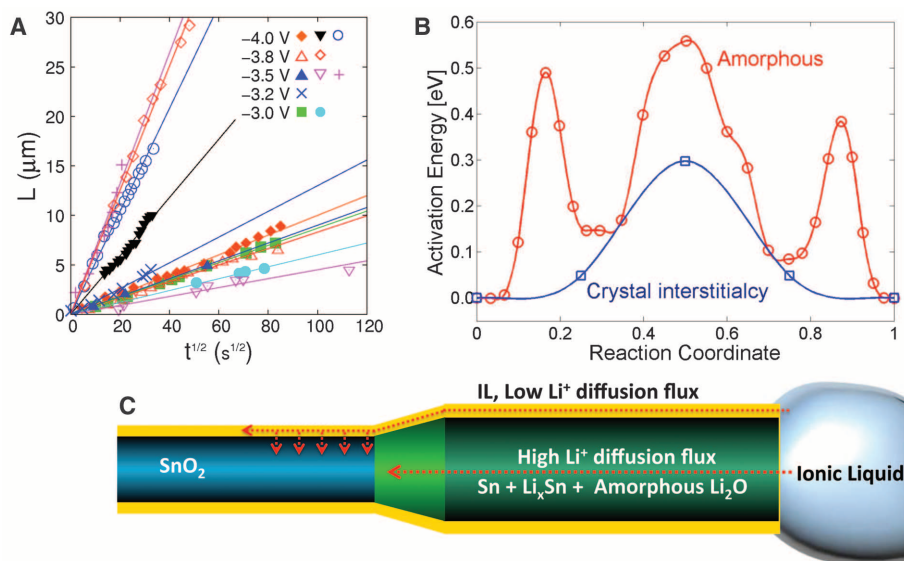


Fig. 4. (A) Plot of the reaction front migration distance L versus the square root of time for 11 nanowires. (B) Representative Li^+ migration energy barrier in crystalline and amorphous Li_2O from DFT calculations. (C) Schematic drawing showing the high Li diffusion flux in Li_2O .

straints at the ACI, large in-plane stresses develop that drive mechanical plasticity. The net shape change of the nanowire observed is therefore not due to lithiation alone, but is the combined outcome of electrochemical-mechanical actions, where stress-induced plasticity plays an important part (fig. S5). These shape-change features mean that the design and packaging of nanowire nanobatteries must take into account the large conformation changes of the nanowire (buckling, coiling, and twisting) without breaking electrical contact or shorting across electrodes. It is also noteworthy that among the several nanowires we charged and discharged, none of them fractured despite the large strain and conformational changes. This is further testimony to the mechanical robustness associated with the nanowire geometry relative to bulk ceramic electrodes (13, 14).

The displacement of the reaction front versus the square root of reaction time is plotted in Fig. 4A using the results of 11 experiments. The nearly parabolic behavior indicates the importance of long-range Li^+ diffusion (Fig. 4C). On the basis of our data, the diffusivity of Li^+ in the reacted amorphous sections ranges from 5×10^{-16} to $5 \times 10^{-14} \text{ m}^2/\text{s}$, which is of the same range as the results reported for bulk Li_2O (29–31) if extrapolated to room temperature. The somewhat large scattering in diffusivity value is reasonable, because unlike a crystal, the amorphous atomic structure is not unique, and slightly different formation conditions (for example, due to different local voltage) of the amorphized nanowire can lead to different diffusivities. The characteristic migration energy barrier of $\sim 0.4 \text{ eV}$ (Fig. 4B), obtained from our ab initio calculations using an ensemble of Li^+ migration paths in Li_2O with approximately the same initial and final potential energies, matches our experimental diffusivities reasonably well. Note that the wetting layer of ILE on the nanowire

surface is so thin (less than 10 nm) that the flux of Li^+ transported by this layer is outmatched by the flux from solid-state diffusion in the amorphous Li_2O reaction product. This explains why the reaction occurred along the longitudinal direction rather than along the radial direction.

To imitate the scenario in a real battery configuration, we have also conducted experiments with the nanowire partially immersed in the ILE to see whether there is any difference in the post-charging shape changes between the immersed segments and the exposed segments of the nanowire (fig. S6). We found no essential difference in the final shape between the two different segments of the nanowire, both of which show large shape changes with extensive buckling and spiraling (fig. S6).

After charging, we also performed discharge, and TEM showed that the Li_xSn alloy nanoparticles were converted back to pure Sn (fig. S7) and that the diameter of the nanowire decreased. The overall volume change during discharge was much less than during the initial charging process, however. During initial charging, the formation of Li_2O caused large volume expansion [irreversible (16)], whereas in the discharging process, the Li_2O glass did not participate in the electrochemical reaction and only the Li_xSn nanoprecipitates, which occupy smaller volume, were active. Although the successful charging and discharging demonstrate that this system constitutes a working electrochemical device (32), we were unable to quantify the reversible capacity of this device because the low discharge current (estimated to be less than 3 pA) was much lower than our noise floor for our electrical current measurement.

The methodology described above should stimulate real-time studies of the microscopic processes in batteries and lead to a more complete understanding of the mechanisms governing battery performance and reliability, especially those

properties that are controlled by microstructure. Although the work was carried out using SnO_2 nanowires, these experiments can be extended to other materials, for either cathode or anode studies. Further, autonomous nanomachines such as nanorobots (33) call for extreme miniaturization of power supplies (34) with energy generation (35) and energy storage functions. The concept of a stand-alone rechargeable nanobattery that uses individual nanowires as electrodes and a nanoscale electrolyte is quite appealing. Although our work falls short of realizing a fully packaged nanobattery, we believe that the in situ characterization and modeling reported here is an important step toward achieving that goal.

References and Notes

- P. Limthongkul, Y. I. Jang, N. J. Dudney, Y. M. Chiang, *Acta Mater.* **51**, 1103 (2003).
- A. K. Padhi, K. S. Nanjundaswamy, J. B. Goodenough, *J. Electrochem. Soc.* **144**, 1188 (1997).
- J. M. Tarascon, M. Armand, *Nature* **414**, 359 (2001).
- Y. Shao-Horn, L. Croguennec, C. Delmas, E. C. Nelson, M. A. O'Keefe, *Nat. Mater.* **2**, 464 (2003).
- B. Kang, G. Ceder, *Nature* **458**, 190 (2009).
- W. Lai et al., *Adv. Mater.* **22**, E139 (2010).
- K. T. Nam et al., *Science* **312**, 885 (2006); 10.1126/science.1122716.
- M. S. Park et al., *Angew. Chem. Int. Ed.* **46**, 750 (2007).
- C. K. Chan et al., *Nat. Nanotechnol.* **3**, 31 (2008).
- H. Kim, J. Cho, *Nano Lett.* **8**, 3688 (2008).
- Y. D. Ko, J. G. Kang, J. G. Park, S. Lee, D. W. Kim, *Nanotechnology* **20**, 455701 (2009).
- A. Magasinski et al., *Nat. Mater.* **9**, 353 (2010).
- T. K. Bhandakkar, H. J. Gao, *Int. J. Solids Struct.* **47**, 1424 (2010).
- T. Zhu, J. Li, *Prog. Mater. Sci.* **55**, 710 (2010).
- D. R. Liu, D. B. Williams, *Philos. Mag. B* **53**, 1123 (1986).
- I. A. Courtney, J. R. Dahn, *J. Electrochem. Soc.* **144**, 2045 (1997).
- H. Gabrisch, R. Yazami, B. Fultz, *Electrochem. Solid State Lett.* **5**, A111 (2002).
- Y. M. Wang, J. Li, A. V. Hamza, T. W. Barbee Jr., *Proc. Natl. Acad. Sci. U.S.A.* **104**, 11155 (2007).
- Z. W. Shan et al., *Phys. Rev. B* **77**, 155419 (2008).
- M. Legros, G. Dehm, E. Arzt, T. J. Balk, *Science* **319**, 1646 (2008).
- D. Wolf, P. R. Okamoto, S. Yip, J. F. Lutsko, M. Kluge, *J. Mater. Res.* **5**, 286 (1990).
- H. J. Fecht, *Nature* **356**, 133 (1992).
- H. Bakker, G. F. Zhou, H. Yang, *Prog. Mater. Sci.* **39**, 159 (1995).
- C. Suryanarayana, *Prog. Mater. Sci.* **46**, 1 (2001).
- J. Y. Huang, H. Yasuda, H. Mori, *Philos. Mag. Lett.* **79**, 305 (1999).
- J. Y. Huang, Y. T. Zhu, X. Z. Liao, R. Z. Valiev, *Philos. Mag. Lett.* **84**, 183 (2004).
- H. Mughrabi, *Philos. Mag.* **86**, 4037 (2006).
- A. G. Khachatryan, *Theory of Structural Transformations in Solids* (Wiley, New York, 1983).
- H. Ohno et al., *J. Nucl. Mater.* **118**, 242 (1983).
- T. Oda, S. Tanaka, *J. Nucl. Mater.* **386–388**, 1087 (2009).
- J. Habasaki, Y. Hiwatari, *Phys. Rev. B* **69**, 144207 (2004).
- A. Brazier et al., *Chem. Mater.* **20**, 2352 (2008).
- K. Kostarelos, *Nanomedicine* **5**, 341 (2010).
- A. E. Curtright, P. J. Bouwman, R. C. Wartena, K. E. Swider-Lyons, *Int. J. Nanotechnol.* **1**, 226 (2004).
- Z. L. Wang, J. Song, *Science* **312**, 242 (2006).
- J.Y.H. thanks K. Xu for valuable discussions. Supported by a Laboratory Directed Research and Development (LDRD) project at Sandia National Laboratories (SNL) and by the Science of Precision Multifunctional Nanostructures for Electrical Energy Storage (NEES), an Energy Frontier Research

Center funded by the U.S. Department of Energy (DOE), Office of Science, Office of Basic Energy Sciences (BES) under award DESC0001160. This work was performed in part at the Sandia-Los Alamos Center for Integrated Nanotechnologies (CINT), a U.S. DOE, Office of BES user facility. The LDRD supported the development and fabrication of platforms and the development of TEM techniques. The NEES center supported some of the additional platform development and fabrication and materials characterization. CINT supported the TEM capability and the fabrication capabilities that were used for the TEM characterization, and this work represents the efforts of several CINT users, primarily those with affiliation external to SNL. SNL is a multiprogram laboratory operated by Sandia Corporation, a wholly owned subsidiary of

Lockheed Martin company, for the DOE's National Nuclear Security Administration under contract DE-AC04-94AL85000. The work of C.M.W. and W.X. was supported by the DOE Office of Science, Offices of Biological and Environmental Research, and was conducted in the Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by DOE's Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory, which is operated by Battelle for the DOE under contract DE-AC05-76RL01830. L.Q., A.K., and J.L. were supported by Honda Research Institute USA, Xi'an Jiaotong University, NSF grants CMMI-0728069, DMR-1008104, and DMR-0520020, and Air Force Office of Scientific Research grant FA9550-08-1-0325. S.X.M., L.Z., and L.Q.Z. were supported by NSF grants

CMMI0825842 and CMMI0928517 through the University of Pittsburgh and SNL. L.Q.Z. thanks the Chinese Scholarship Council for financial support and Z. Ye's encouragement from Zhejiang University.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1515/DC1
Materials and Methods
Figs. S1 to S11
Movies S1 to S5
References

26 July 2010; accepted 26 October 2010
10.1126/science.1195628

Optomechanically Induced Transparency

Stefan Weis,^{1,2*} Rémi Rivière,^{2*} Samuel Deléglise,^{1,2*} Emanuel Gavartin,¹ Olivier Arcizet,³ Albert Schliesser,^{1,2} Tobias J. Kippenberg^{1,2†}

Electromagnetically induced transparency is a quantum interference effect observed in atoms and molecules, in which the optical response of an atomic medium is controlled by an electromagnetic field. We demonstrated a form of induced transparency enabled by radiation-pressure coupling of an optical and a mechanical mode. A control optical beam tuned to a sideband transition of a micro-optomechanical system leads to destructive interference for the excitation of an intracavity probe field, inducing a tunable transparency window for the probe beam. Optomechanically induced transparency may be used for slowing and on-chip storage of light pulses via microfabricated optomechanical arrays.

Coherent interaction of laser radiation with multilevel atoms and molecules can lead to quantum interference in the electronic excitation pathways (1). A prominent example observed in atomic three-level systems is the phenomenon of electromagnetically induced transparency (EIT), in which a control laser induces a narrow spectral transparency window for a weak probe laser beam. When this generic EIT effect had first been observed in an atomic gas (2), its relevance in nonlinear optics and optical (quantum) information processing was quickly recognized. In particular, the rapid variation of the refractive index concomitant with the opening of the transparency window gives rise to a dramatic reduction of the group velocity of a propagating optical pulse (3, 4). Dynamic control of EIT with the control laser enables even a complete stop, that is, storage, of the pulse in an atomic medium (5, 6). The experimental demonstration of slowing and stopping light (3–6) has attracted strong attention, because it provides a route to implement a photonic quantum memory (7) or a classical optical buffer. EIT has subsequently been studied in a wide variety of atomic media, but also in several solid-state systems (8, 9) with a well-suited level structure.

Recent experiments with optomechanical systems have demonstrated that the mechanical re-

sponse to thermal forces can be controlled by an optical field. This effect has been exploited, for example, to implement optomechanical laser cooling and amplification (10–13) as well as normal mode splitting (14). In other work, the mechanical response was optically tailored to exhibit destructive interference between different mechanical excitation pathways (15). Whereas in these studies, the mechanical response to thermal Langevin force was modified, we demonstrate here, as recently suggested (16, 17), that the system's optical response to a weak "probe" laser can be controlled by a second "control" laser driving the lower motional sideband. A window of transparency arises from the destructive interference of excitation pathways for the intracavity probe field when a two-photon resonance condition is met. As pointed out independently, this effect can be considered a strict optomechanical analog of EIT (18), originating from a similar effective interaction Hamiltonian (19). Advantageously, this form of induced transparency does not rely on naturally occurring resonances and could therefore also be applied to previously inaccessible wavelength regions such as the technologically important near-infrared. Furthermore, a single optomechanical element can already achieve unity contrast, which in the atomic case is only possible within the setting of cavity quantum electrodynamics (20).

Our experiment (Fig. 1) consists of an optomechanical system featuring linear optomechanical coupling G in the sense that the cavity resonance frequency is given by $\omega'_c(x) = \omega_c + Gx$, where ω_c is the unperturbed resonance frequency. A control laser (frequency ω_l) maintains

a control field $\bar{a}e^{-i\omega_l t}$, containing $|\bar{a}|^2$ photons, in the cavity. The static radiation pressure originating from this field displaces the mechanical mode by $\bar{x}$, leading to an effective detuning from the cavity resonance $\bar{\Delta} = \omega_l - (\omega_c + G\bar{x})$. We consider the situation where the control laser is tuned close to the lower motional sideband, i.e., $\bar{\Delta} \approx -\Omega_m$, where Ω_m is the mechanical (angular) resonance frequency. A second, weak laser oscillating at $\omega_p = \omega_l + \Omega$, is subsequently used to probe the (modified) cavity resonance by driving an intracavity probe field contained in a perturbation term $\delta a(t)$.

In the case of a weak probe field (compared to the control field), one can linearize the optomechanical dynamics (21) for the mechanical displacement $x(t) = \bar{x} + \delta x(t)$ and the intracavity field $a(t) = [\bar{a} + \delta a(t)]e^{-i\omega_l t}$ around the steady-state values $(\bar{x}, \bar{a})$. For the probe power transmission—that is, the ratio of the probe power returned from the system divided by the input probe power—the general expression

$$|t_p|^2 = \left| 1 - \frac{1 + if(\Omega)}{-i(\bar{\Delta} + \Omega) + \kappa/2 + 2\bar{\Delta}f(\Omega)} \eta_c \kappa \right|^2 \quad (1)$$

with

$$f(\Omega) = \hbar G^2 \bar{a}^2 \frac{\chi(\Omega)}{i(\bar{\Delta} - \Omega) + \kappa/2} \quad (2)$$

can be derived [see (16–18) and supporting online material (SOM) Eq. S25]. Here, $\chi(\Omega) = [m_{\text{eff}}(\Omega_m^2 - \Omega^2 - i\Gamma_m\Omega)]^{-1}$ is the susceptibility of the mechanical oscillator of effective mass m_{eff} and damping rate Γ_m . The optical mode is characterized by a total loss rate $\kappa = \kappa_0 + \kappa_{\text{ex}}$ and the cavity coupling parameter $\eta_c = \kappa_{\text{ex}}/(\kappa_0 + \kappa_{\text{ex}})$. The presence of a control field $\bar{a}$ (tuned to the lower sideband) induces a transmission window for the probe beam when the resonance condition $\Omega \approx \Omega_m$ is met (Fig. 1). The depth and the width of this transmission window are tunable by the power of the control beam, as in the case of atomic EIT, with the best contrast achieved in the case of critical coupling $\eta_c = 1/2$.

To gain more physical insight into this phenomenon, it is instructive to consider this effect in a sideband picture. The simultaneous presence of control and probe fields generates a radiation-

¹Ecole Polytechnique Fédérale de Lausanne, EPFL, 1015 Lausanne, Switzerland. ²Max-Planck-Institut für Quantenoptik, Hans-Kopfermann-Strasse 1, 85748 Garching, Germany. ³Institut Néel, 25 Rue des Martyrs, 38042 Grenoble, France.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: tobias.kippenberg@epfl.ch

Center funded by the U.S. Department of Energy (DOE), Office of Science, Office of Basic Energy Sciences (BES) under award DESC0001160. This work was performed in part at the Sandia-Los Alamos Center for Integrated Nanotechnologies (CINT), a U.S. DOE, Office of BES user facility. The LDRD supported the development and fabrication of platforms and the development of TEM techniques. The NEES center supported some of the additional platform development and fabrication and materials characterization. CINT supported the TEM capability and the fabrication capabilities that were used for the TEM characterization, and this work represents the efforts of several CINT users, primarily those with affiliation external to SNL. SNL is a multiprogram laboratory operated by Sandia Corporation, a wholly owned subsidiary of

Lockheed Martin company, for the DOE's National Nuclear Security Administration under contract DE-AC04-94AL85000. The work of C.M.W. and W.X. was supported by the DOE Office of Science, Offices of Biological and Environmental Research, and was conducted in the Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by DOE's Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory, which is operated by Battelle for the DOE under contract DE-AC05-76RL01830. L.Q., A.K., and J.L. were supported by Honda Research Institute USA, Xi'an Jiaotong University, NSF grants CMMI-0728069, DMR-1008104, and DMR-0520020, and Air Force Office of Scientific Research grant FA9550-08-1-0325. S.X.M., L.Z., and L.Q.Z. were supported by NSF grants

CMMI0825842 and CMMI0928517 through the University of Pittsburgh and SNL. L.Q.Z. thanks the Chinese Scholarship Council for financial support and Z. Ye's encouragement from Zhejiang University.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1515/DC1
Materials and Methods
Figs. S1 to S11
Movies S1 to S5
References

26 July 2010; accepted 26 October 2010
10.1126/science.1195628

Optomechanically Induced Transparency

Stefan Weis,^{1,2,*} Rémi Rivière,^{2,*} Samuel Deléglise,^{1,2,*} Emanuel Gavartin,¹ Olivier Arcizet,³ Albert Schliesser,^{1,2} Tobias J. Kippenberg^{1,2,†}

Electromagnetically induced transparency is a quantum interference effect observed in atoms and molecules, in which the optical response of an atomic medium is controlled by an electromagnetic field. We demonstrated a form of induced transparency enabled by radiation-pressure coupling of an optical and a mechanical mode. A control optical beam tuned to a sideband transition of a micro-optomechanical system leads to destructive interference for the excitation of an intracavity probe field, inducing a tunable transparency window for the probe beam. Optomechanically induced transparency may be used for slowing and on-chip storage of light pulses via microfabricated optomechanical arrays.

Coherent interaction of laser radiation with multilevel atoms and molecules can lead to quantum interference in the electronic excitation pathways (1). A prominent example observed in atomic three-level systems is the phenomenon of electromagnetically induced transparency (EIT), in which a control laser induces a narrow spectral transparency window for a weak probe laser beam. When this generic EIT effect had first been observed in an atomic gas (2), its relevance in nonlinear optics and optical (quantum) information processing was quickly recognized. In particular, the rapid variation of the refractive index concomitant with the opening of the transparency window gives rise to a dramatic reduction of the group velocity of a propagating optical pulse (3, 4). Dynamic control of EIT with the control laser enables even a complete stop, that is, storage, of the pulse in an atomic medium (5, 6). The experimental demonstration of slowing and stopping light (3–6) has attracted strong attention, because it provides a route to implement a photonic quantum memory (7) or a classical optical buffer. EIT has subsequently been studied in a wide variety of atomic media, but also in several solid-state systems (8, 9) with a well-suited level structure.

Recent experiments with optomechanical systems have demonstrated that the mechanical re-

sponse to thermal forces can be controlled by an optical field. This effect has been exploited, for example, to implement optomechanical laser cooling and amplification (10–13) as well as normal mode splitting (14). In other work, the mechanical response was optically tailored to exhibit destructive interference between different mechanical excitation pathways (15). Whereas in these studies, the mechanical response to thermal Langevin force was modified, we demonstrate here, as recently suggested (16, 17), that the system's optical response to a weak "probe" laser can be controlled by a second "control" laser driving the lower motional sideband. A window of transparency arises from the destructive interference of excitation pathways for the intracavity probe field when a two-photon resonance condition is met. As pointed out independently, this effect can be considered a strict optomechanical analog of EIT (18), originating from a similar effective interaction Hamiltonian (19). Advantageously, this form of induced transparency does not rely on naturally occurring resonances and could therefore also be applied to previously inaccessible wavelength regions such as the technologically important near-infrared. Furthermore, a single optomechanical element can already achieve unity contrast, which in the atomic case is only possible within the setting of cavity quantum electrodynamics (20).

Our experiment (Fig. 1) consists of an optomechanical system featuring linear optomechanical coupling G in the sense that the cavity resonance frequency is given by $\omega'_c(x) = \omega_c + Gx$, where ω_c is the unperturbed resonance frequency. A control laser (frequency ω_l) maintains

a control field $\bar{a}e^{-i\omega_l t}$, containing $|\bar{a}|^2$ photons, in the cavity. The static radiation pressure originating from this field displaces the mechanical mode by $\bar{x}$, leading to an effective detuning from the cavity resonance $\bar{\Delta} = \omega_l - (\omega_c + G\bar{x})$. We consider the situation where the control laser is tuned close to the lower motional sideband, i.e., $\bar{\Delta} \approx -\Omega_m$, where Ω_m is the mechanical (angular) resonance frequency. A second, weak laser oscillating at $\omega_p = \omega_l + \Omega$, is subsequently used to probe the (modified) cavity resonance by driving an intracavity probe field contained in a perturbation term $\delta a(t)$.

In the case of a weak probe field (compared to the control field), one can linearize the optomechanical dynamics (21) for the mechanical displacement $x(t) = \bar{x} + \delta x(t)$ and the intracavity field $a(t) = [\bar{a} + \delta a(t)]e^{-i\omega_l t}$ around the steady-state values $(\bar{x}, \bar{a})$. For the probe power transmission—that is, the ratio of the probe power returned from the system divided by the input probe power—the general expression

$$|t_p|^2 = \left| 1 - \frac{1 + if(\Omega)}{-i(\bar{\Delta} + \Omega) + \kappa/2 + 2\bar{\Delta}f(\Omega)} \eta_c \kappa \right|^2 \quad (1)$$

with

$$f(\Omega) = \hbar G^2 \bar{a}^2 \frac{\chi(\Omega)}{i(\bar{\Delta} - \Omega) + \kappa/2} \quad (2)$$

can be derived [see (16–18) and supporting online material (SOM) Eq. S25]. Here, $\chi(\Omega) = [m_{\text{eff}}(\Omega_m^2 - \Omega^2 - i\Gamma_m\Omega)]^{-1}$ is the susceptibility of the mechanical oscillator of effective mass m_{eff} and damping rate Γ_m . The optical mode is characterized by a total loss rate $\kappa = \kappa_0 + \kappa_{\text{ex}}$ and the cavity coupling parameter $\eta_c = \kappa_{\text{ex}}/(\kappa_0 + \kappa_{\text{ex}})$. The presence of a control field $\bar{a}$ (tuned to the lower sideband) induces a transmission window for the probe beam when the resonance condition $\Omega \approx \Omega_m$ is met (Fig. 1). The depth and the width of this transmission window are tunable by the power of the control beam, as in the case of atomic EIT, with the best contrast achieved in the case of critical coupling $\eta_c = 1/2$.

To gain more physical insight into this phenomenon, it is instructive to consider this effect in a sideband picture. The simultaneous presence of control and probe fields generates a radiation-

¹Ecole Polytechnique Fédérale de Lausanne, EPFL, 1015 Lausanne, Switzerland. ²Max-Planck-Institut für Quantenoptik, Hans-Kopfermann-Strasse 1, 85748 Garching, Germany. ³Institut Néel, 25 Rue des Martyrs, 38042 Grenoble, France.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: tobias.kippenberg@epfl.ch

pressure force oscillating at the frequency difference Ω . If this driving force oscillates close to the mechanical resonance frequency Ω_m , the mechanical mode starts to oscillate coherently, $\delta x(t) = 2\text{Re}[X e^{-i\Omega t}]$. This in turn gives rise to Stokes- and anti-Stokes scattering of light from the strong intracavity control field. If the system resides deep enough in the resolved-sideband (RSB) regime with $\kappa \ll \Omega_m$, Stokes scattering (to the optical frequency $\omega_l - \Omega$) is strongly suppressed because it is highly off-resonant with the optical cavity. We can therefore assume that only an anti-Stokes field builds up inside the cavity, $\delta a(t) \approx A^- e^{-i\Omega t}$. However, this field of frequency $\omega_p = \omega_l + \Omega$ is degenerate with the near-resonant probe field sent to the cavity. Destructive interference of these two driving waves can suppress the build-up of an intracavity probe field. These processes are captured by the Langevin equations of motion for the complex amplitudes A^- and X , which require in the steady state (SOM Eqs. S26 and S27)

$$(-i\Delta' + \kappa/2)A^- = -iG\bar{a}X + \sqrt{\eta_c\kappa} \delta s_{in} \quad (3)$$

$$2m_{\text{eff}}\Omega_m(-i\Delta' + \Gamma_m/2)X = -ihG\bar{a}A^- \quad (4)$$

where δs_{in} is the amplitude of the probe field drive, and we abbreviate $\Delta' \equiv \Omega - \Omega_m$. We have assumed a high-quality factor of the mechanical oscillator ($\Gamma_m \ll \Omega_m$) and the control beam detuning $\bar{\Delta} = -\Omega_m$. The solution for the intracavity probe field amplitude reads

$$A^- = \frac{\sqrt{\eta_c\kappa}}{(-i\Delta' + \kappa/2) + \frac{\Omega_c^2/4}{-i\Delta' + \Gamma_m/2}} \delta s_{in} \quad (5)$$

This solution has a form well known from the response of an EIT medium to a probe field (1). The coherence between the two ground states of an atomic Λ system, and the coherence between the levels probed by the probe laser undergo the same evolution as do the mechanical oscillation amplitude and the intracavity probe field in the case of optomechanically induced transparency (OMIT). The role of the control laser's Rabi frequency in an atomic system is taken by the optomechanical coupling rate $\Omega_c = 2\bar{a}Gx_{\text{zpf}}$, where $x_{\text{zpf}} = \sqrt{\hbar/2m_{\text{eff}}\Omega_m}$ designates the spread of the ground-state wave function of the mechanical oscillator. For $\Omega_c > \Gamma_m$, κ the system enters the strong coupling regime (22, 23) investigated re-

cently in the mechanical domain (14), in which the optical and mechanical systems are hybridized to dressed states that differ by $\hbar\Omega_c$ in their energy.

OMIT is realized using toroidal whispering-gallery-mode microresonators (Fig. 2A) (10, 17). The cavity is operated in the undercoupled regime ($\eta_c < 1/2$), which together with modal coupling between counterpropagating modes (SOM Sec. 7) leads to a nonzero probe (amplitude) transmission $t_r = t_p(\Delta' = 0, \Omega_c = 0)$ at resonance (Fig. 2B), even in the absence of the control beam. In the case of the present device, $|t_r|^2 \approx 0.5$. [Note, however, that $|t_r|^2 < 0.01$ can be achieved with silica toroids (24).] To separate the effects of this residual transmission from OMIT, we introduce the normalized transmission of the probe $t'_p = (t_p - t_r)/(1 - t_r)$.

The mechanical motion was detected using a balanced homodyne detection scheme (fig. S1) measuring the phase quadrature of the field emerging from the cavity (25). This allows extracting the parameters of the device used in these experiments, which are given by ($m_{\text{eff}}, G/2\pi, \Gamma_m/2\pi, \Omega_m/2\pi, \kappa/2\pi$) $\approx$ (20 ng, -12 GHz/nm, 41 kHz, 51.8 MHz, 15 MHz), placing it well into the resolved sideband regime (25). To probe the cavity transmission spectrum in the presence of a control beam, the Ti:sapphire control laser is frequency modulated at frequency Ω using a broadband phase modulator, creating two side-

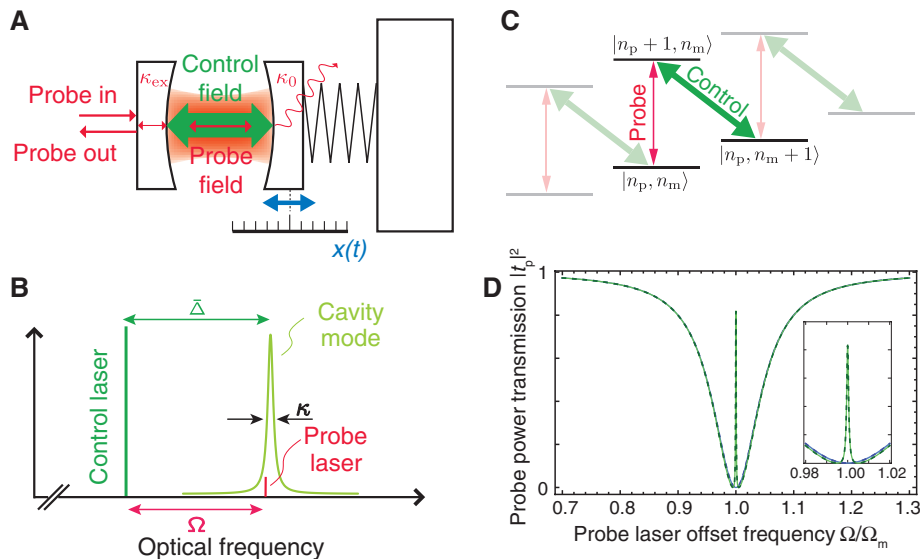


Fig. 1. Optomechanically induced transparency. **(A)** A generic optomechanical system consists of an optical cavity with a movable boundary, illustrated here as a Fabry-Perot-type resonator in which one mirror acts like a mass-on-a-spring movable along x . The cavity has an intrinsic photon loss rate κ_0 and is coupled to an external propagating mode at the rate κ_{ex} . Through the external mode, the resonator is populated with a control field (only intracavity field is shown). The response of this driven optomechanical system is probed by a weak probe field sent toward the cavity, the transmission of which (i.e., the returned field “Probe out”) is analyzed here. **(B)** The frequency of the control field is detuned by $\bar{\Delta}$ from the cavity resonance frequency, where a detuning close to the lower mechanical sideband, $\bar{\Delta} \approx -\Omega_m$, is chosen. The probe laser’s frequency is offset by the tunable radio frequency Ω from the control laser. The dynamics of interest occur when the probe laser is tuned over the optical resonance of the cavity, which has a linewidth of $\kappa = \kappa_0 + \kappa_{\text{ex}}$. **(C)** Level scheme of the optomechanical system. The control field is tuned close to red-sideband transitions, in which a mechanical excitation quantum is annihilated (mechanical occupation $n_m \rightarrow n_m - 1$) when a photon is added to the cavity (optical occupation $n_p \rightarrow n_p + 1$), therefore coupling the corresponding energy eigenstates. The probe field probes transitions in which the mechanical oscillator occupation is unchanged. **(D)** Transmission of the probe laser power through the optomechanical system in the case of a critically coupled cavity $\kappa_0 = \kappa_{\text{ex}}$ as a function of normalized probe laser frequency offset, when the control field is off (blue lines) and on (green lines). Dashed and full lines correspond to the models based on the full (Eq. 1) and approximative (Eq. 5) calculations, respectively.

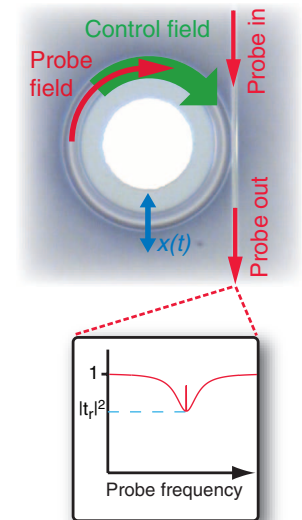
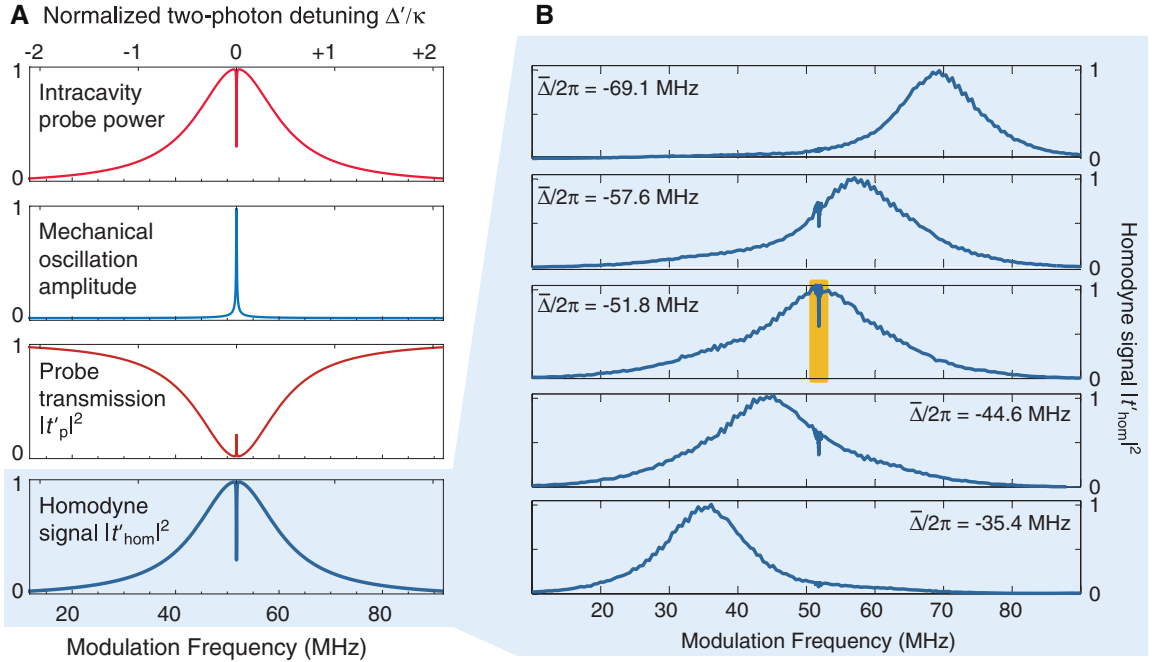


Fig. 2. Optomechanical system. **(Top)** A toroidal microcavity is used to demonstrate OMIT: The resonator is coupled to the control and probe fields using a tapered fiber. The optical mode couples through radiation pressure force to the mechanical radial breathing mode of the structure. In this ring geometry, the cavity transmission, defined by the ratio of the returned probe-field amplitude divided by the incoming probe field is simply given by the transmission through the tapered fiber. **(Bottom)** Under the chosen waveguide-toroid coupling conditions, there is a nonzero probe power transmission $|t_r|^2$ at resonance. The control field induces an additional transparency window with a contrast up to $1 - |t_r|^2$.

Fig. 3. Observation of OMIT. **(A)** Theoretically expected intracavity probe power, oscillation amplitude X , normalized probe power transmission $|t'_p|^2$, and the normalized homodyne signal $|t'_{\text{hom}}|^2$ as a function of the modulation frequency $\Omega/2\pi$ (top to bottom panels). The first two panels have additionally been normalized to unity. When the two-photon resonance condition $\Delta' = 0$ is met, the mechanical oscillator is excited, giving rise to destructive interference of excitation pathways for an intracavity probe field. The probe transmission therefore exhibits an inverted dip, which can be easily identified in the homodyne signal. **(B)** Experimentally observed normalized homodyne traces when the probe frequency is scanned by sweeping the phase modulator frequency Ω for different values of control beam detuning $\bar{\Delta}$. Whereas the center of the response of the bare optical cavity shifts correspondingly, the sharp dip characteristic of OMIT occurs always for $\Delta' = 0$. The power of the control



beam sent to the cavity is 0.5 mW in these measurements. The middle panel shows the operating conditions where the control beam is tuned to the lower motional sideband $\bar{\Delta} \approx -\Omega_m = -2\pi \cdot 51.8$ MHz. The region around the central dip (orange background) is studied in more detail in a dedicated experimental series (see Fig. 4).

bands at $\omega_1 + \Omega = \omega_p$ (probe field) and $\omega_1 - \Omega$. In the resolved sideband regime, only the upper sideband, nearly resonant, interacts with the optomechanical system. Keeping the laser detuned to the lower motional sideband of the cavity ($\bar{\Delta} \approx -\Omega_m$), a sweep of the modulation frequency Ω scans the probe field through the cavity resonance. As shown in detail in the SOM (Sec. 5), demodulation of the total homodyne signal at the modulation frequency Ω using a network analyzer (NA) allows extracting a “transmission” homodyne signal t_{hom} , which, in the RSB regime, is related to the probe transmission by the simple relation $t_{\text{hom}} \approx 1 - t_p$.

Figure 3A shows the theoretically expected response of the optomechanical system and the detected signals. Clearly, the OMIT window is apparent in the intracavity probe power as described by Eq. 5, occurring simultaneously with the onset of radiation-pressure-driven mechanical oscillations. The excitation of the intracavity probe field therefore is suppressed, and the transmitted field nearly equals the probe field sent to the cavity. The lowest panel shows the corresponding homodyne signal, and the five panels in Fig. 3B show experimentally measured homodyne traces for detunings $\bar{\Delta}/2\pi$ varying from -69.1 MHz to -35.4 MHz and a control laser power of 0.5 mW. The center of the probe extinction (maximum of the homodyne signal) always occurs for $\Omega \approx -\bar{\Delta}$, because the probe laser then matches the cavity resonance [$\omega_p \approx \omega'_c(\bar{x})$]. Notably, however, the sharp OMIT window occurs only when the two-photon resonance con-

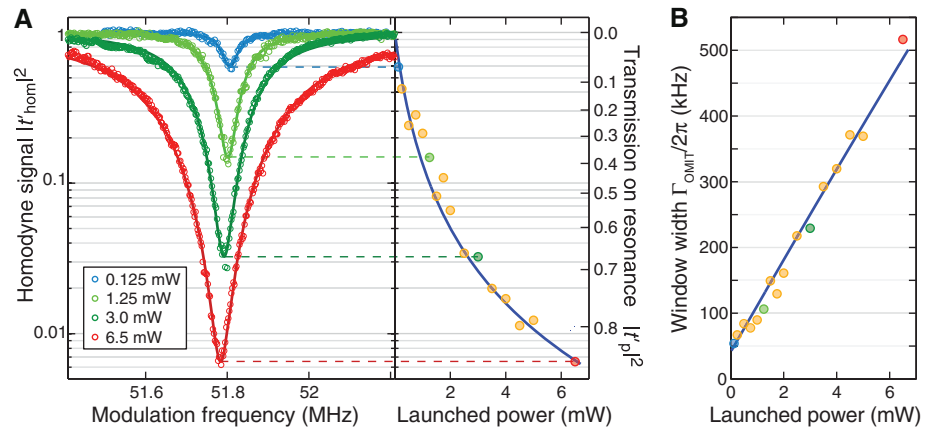


Fig. 4. Controlling OMIT. **(A)** Experimental normalized homodyne traces in the presence of a control beam (circles) for four different powers in the control beam from 0.125 mW up to 6.5 mW, and Lorentzian models. The minimum homodyne signal (measured at $\Delta' = 0$) directly indicates the maximum probe power transmission achieved in this case. These values are given in the right panel for a larger set of probe scans, together with the theoretical model developed in this work. **(B)** Width of the transparency window extracted from the same set of probe scans. Good agreement with the theoretical prediction is found over the entire power range.

dition $\Omega = \Omega_m$ (with $\Omega = \omega_p - \omega_1$) is met, independent of the detuning $\bar{\Delta}$ of the control beam, giving clear evidence to the theoretically suggested underlying mechanism.

To analyze the effect of the control beam more systematically, its detuning was fixed to the lower motional sideband. Varying its power from 0.125 to 6.5 mW, traces of the homodyne signal are taken in the vicinity of the two-photon

resonance (Fig. 4). Dips of increasing depth and width are observed, which can be modeled by a simple Lorentzian function (SOM Sec. 3). The minimum homodyne signal is obtained under the condition of the two-photon resonance $\Delta' = 0$. In this case, the homodyne signal power and the probe power transmission are simply interrelated by $|t'_p|^2 = (1 - |t'_{\text{hom}}|)^2$, where $t'_{\text{hom}} = t_{\text{hom}}/(1 - t_r)$ is the normalized homodyne signal. The width

of the measured dip in the normalized homodyne signal is equal to that of the coupling-induced transmission window $\Gamma_{\text{OMIT}} \approx \Gamma_m (1 + C)$, where $C \equiv \Omega_c^2/\Gamma_m \kappa$ is an equivalent optomechanical cooperativity parameter (14). From the model (Eq. 5), the expected probe transmission on resonance is simply given by $t_p' (\Delta' = 0) = C/(C + 1)$. Our data match these expectations well if we allow for a linear correction factor in the optomechanical coupling frequency Ω_c due to modal coupling (SOM Sec. 7) and losses in the fiber taper. We have reached probe power transmission $|t_p'|^2$ up to 81%, indicating the high contrast achievable in OMIT.

In fact, any optomechanical system reaching $C \geq 1$ can realize an appreciable control-induced probe transmission, as desired, for example, in all-optical switches. Interestingly, the systems currently available reach $C \approx 1$ with only thousands (26) or even hundreds (27) of control photons in the cavity, and recently emerging integrated nano-optomechanical structures (28) may be able to further reduce this number. The resulting extreme optical nonlinearities could be of interest for both fundamental and applied studies.

The tunable probe transmission window also modifies the propagation of a probe pulse due to the variation of the complex phase picked by its different frequency components. Indeed, a resonant probe pulse experiences a group delay of $\tau_g \approx 2/\Gamma_{\text{OMIT}}$ in the regime $C \gtrsim 1$ of interest (SOM Sec. 6), a value exceeding several seconds in some available optomechanical systems (29). However, undistorted pulse propagation only occurs if the full probe-pulse spectrum is contained within the transparency window of the system. This restricts the effectiveness of such a delay due to the fixed delay-bandwidth product of $\tau_g \Gamma_{\text{OMIT}} \approx 2$. A cascade of systems may alleviate

this shortcoming—the most interesting scenario being a large array of concatenated optomechanical systems, as suggested in the context of OMIT (16, 17, 30), and radio frequency/microwave photonics (15). The group delay could then be dynamically tuned while the probe pulse is propagating through the array. Such systems could be practically implemented in lithographically designed optomechanical systems both in the microwave (27) and optical (31) domain.

Note added in proof: After online publication of this work, OMIT has also been reported in microwave optomechanical systems by Teufel *et al.* (32).

References and Notes

- M. Fleischhauer, A. Imamoglu, J. P. Marangos, *Rev. Mod. Phys.* **77**, 633 (2005).
- K.-J. Boller, A. Imamoglu, S. E. Harris, *Phys. Rev. Lett.* **66**, 2593 (1991).
- A. Kasapi, M. Jain, G. Y. Yin, S. E. Harris, *Phys. Rev. Lett.* **74**, 2447 (1995).
- L. V. Hau, S. E. Harris, Z. Dutton, C. H. Behroozi, *Nature* **397**, 594 (1999).
- D. F. Phillips, A. Fleischhauer, A. Mair, R. L. Walsworth, M. D. Lukin, *Phys. Rev. Lett.* **86**, 783 (2001).
- C. Liu, Z. Dutton, C. H. Behroozi, L. V. Hau, *Nature* **409**, 490 (2001).
- M. D. Lukin, A. Imamoglu, *Nature* **413**, 273 (2001).
- A. V. Turukhin *et al.*, *Phys. Rev. Lett.* **88**, 023602 (2002).
- D. Brunner *et al.*, *Science* **325**, 70 (2009).
- T. J. Kippenberg, H. Rokhsari, T. Carmon, A. Scherer, K. J. Vahala, *Phys. Rev. Lett.* **95**, 033901 (2005).
- A. Schliesser, P. Del'Haye, N. Nooshi, K. J. Vahala, T. J. Kippenberg, *Phys. Rev. Lett.* **97**, 243905 (2006).
- O. Arcizet, P.-F. Cohadon, T. Briant, M. Pinard, A. Heidmann, *Nature* **444**, 71 (2006).
- S. Gigan *et al.*, *Nature* **444**, 67 (2006).
- S. Gröblacher, K. Hammerer, M. R. Vanner, M. Aspelmeyer, *Nature* **460**, 724 (2009).
- Q. Lin *et al.*, *Nat. Photonics* **4**, 236 (2010).
- A. Schliesser, Cavity optomechanics and optical frequency comb generation with silica whispering-gallery-mode

microresonators, Thesis, Ludwig-Maximilians-Universität München (2009); <http://edoc.ub.uni-muenchen.de/10940>.

- A. Schliesser, T. J. Kippenberg, in *Advances in Atomic, Molecular and Optical Physics*, Vol. 58, E. Arimondo, P. Berman, C. C. Lin, Eds. (Elsevier Academic Press, 2010), pp. 207–323.
- G. S. Agarwal, S. Huang, *Phys. Rev. A* **81**, 041803 (2010).
- J. Zhang, K. Peng, S. L. Braunstein, *Phys. Rev. A* **68**, 013808 (2003).
- M. Mücke *et al.*, *Nature* **465**, 755 (2010).
- C. Fabre *et al.*, *Phys. Rev. A* **49**, 1337 (1994).
- F. Marquardt, J. P. Chen, A. A. Clerk, S. M. Girvin, *Phys. Rev. Lett.* **99**, 093902 (2007).
- J. M. Dobrindt, I. Wilson-Rae, T. J. Kippenberg, *Phys. Rev. Lett.* **101**, 263602 (2008).
- M. Cai, O. Painter, K. J. Vahala, *Phys. Rev. Lett.* **85**, 74 (2000).
- A. Schliesser, O. Arcizet, R. Rivière, G. Anetsberger, T. Kippenberg, *Nat. Phys.* **5**, 509 (2009).
- G. Anetsberger *et al.*, *Nat. Phys.* **5**, 909 (2009).
- C. A. Regal, J. D. Teufel, K. W. Lehnert, *Nat. Phys.* **4**, 555 (2008).
- M. Eichenfield, R. Camacho, J. Chan, K. J. Vahala, O. Painter, *Nature* **459**, 550 (2009).
- J. D. Thompson *et al.*, *Nature* **452**, 72 (2008).
- D. E. Chang, A. H. Safavi-Naeini, M. Hafezi, O. Painter, <http://arxiv.org/abs/1006.3829> (2010).
- M. Eichenfield, J. Chan, R. M. Camacho, K. J. Vahala, O. Painter, *Nature* **462**, 78 (2009).
- J. D. Teufel *et al.*; <http://arxiv.org/abs/1011.3067> (2010).
- T.J.K. acknowledges financial support by the Max Planck Society, European Research Council Starting Grant (SiMP), the European Union's MINOS, a Marie Curie Excellence Grant, the Swiss National Science Foundation, National Centre of Competence and Research of Quantum Photonics, and the Defense Advanced Research Projects Agency. S.D. is funded by a Marie Curie Individual Fellowship.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1195596/DC1
SOM Text

Figs. S1 to S3
References

26 July 2010; accepted 1 November 2010
Published online 11 November 2010;
10.1126/science.1195596

A Determination of the Cloud Feedback from Climate Variations over the Past Decade

A. E. Dessler

Estimates of Earth's climate sensitivity are uncertain, largely because of uncertainty in the long-term cloud feedback. I estimated the magnitude of the cloud feedback in response to short-term climate variations by analyzing the top-of-atmosphere radiation budget from March 2000 to February 2010. Over this period, the short-term cloud feedback had a magnitude of 0.54 ± 0.74 (2σ) watts per square meter per kelvin, meaning that it is likely positive. A small negative feedback is possible, but one large enough to cancel the climate's positive feedbacks is not supported by these observations. Both long- and short-wave components of short-term cloud feedback are also likely positive. Calculations of short-term cloud feedback in climate models yield a similar feedback. I find no correlation in the models between the short- and long-term cloud feedbacks.

Much of the global warming expected over the next century comes from feedbacks rather than direct warming from CO₂ and other greenhouse agents. Of these feedbacks,

the most complex and least understood is the cloud feedback (1, 2). Clouds affect the climate by reflecting incoming solar radiation back to space, which tends to cool the climate, and by trapping

outgoing infrared radiation, which tends to warm the climate. In our present climate, the reflection of solar energy back to space dominates, and the net effect of clouds is to reduce the net flux of incoming energy at the top of the atmosphere (TOA) by ~ 20 W/m², as compared to an otherwise identical planet without clouds. The cloud feedback refers to changes in this net effect of clouds as the planet warms. If, as the climate warms, cloud changes further reduce net incoming energy, this will offset some of the warming, resulting in a negative cloud feedback. If, on the other hand, cloud changes lead to increases in net incoming energy, then the change will amplify the initial warming, resulting in a positive cloud feedback.

Climate models disagree on the magnitude of the cloud feedback, simulating a range of cloud feedbacks in response to long-term global warming from near zero to a positive feedback of 1 W/m²/K (3, 4). This spread is the single most important reason for the large spread in the climate

Department of Atmospheric Sciences, Texas A&M University, College Station, TX, USA. E-mail: adessler@tamu.edu

of the measured dip in the normalized homodyne signal is equal to that of the coupling-induced transmission window $\Gamma_{\text{OMIT}} \approx \Gamma_m (1 + C)$, where $C \equiv \Omega_c^2/\Gamma_m \kappa$ is an equivalent optomechanical cooperativity parameter (14). From the model (Eq. 5), the expected probe transmission on resonance is simply given by $t_p' (\Delta' = 0) = C/(C + 1)$. Our data match these expectations well if we allow for a linear correction factor in the optomechanical coupling frequency Ω_c due to modal coupling (SOM Sec. 7) and losses in the fiber taper. We have reached probe power transmission $|t_p'|^2$ up to 81%, indicating the high contrast achievable in OMIT.

In fact, any optomechanical system reaching $C \geq 1$ can realize an appreciable control-induced probe transmission, as desired, for example, in all-optical switches. Interestingly, the systems currently available reach $C \approx 1$ with only thousands (26) or even hundreds (27) of control photons in the cavity, and recently emerging integrated nano-optomechanical structures (28) may be able to further reduce this number. The resulting extreme optical nonlinearities could be of interest for both fundamental and applied studies.

The tunable probe transmission window also modifies the propagation of a probe pulse due to the variation of the complex phase picked by its different frequency components. Indeed, a resonant probe pulse experiences a group delay of $\tau_g \approx 2/\Gamma_{\text{OMIT}}$ in the regime $C \gtrsim 1$ of interest (SOM Sec. 6), a value exceeding several seconds in some available optomechanical systems (29). However, undistorted pulse propagation only occurs if the full probe-pulse spectrum is contained within the transparency window of the system. This restricts the effectiveness of such a delay due to the fixed delay-bandwidth product of $\tau_g \Gamma_{\text{OMIT}} \approx 2$. A cascade of systems may alleviate

this shortcoming—the most interesting scenario being a large array of concatenated optomechanical systems, as suggested in the context of OMIT (16, 17, 30), and radio frequency/microwave photonics (15). The group delay could then be dynamically tuned while the probe pulse is propagating through the array. Such systems could be practically implemented in lithographically designed optomechanical systems both in the microwave (27) and optical (31) domain.

Note added in proof: After online publication of this work, OMIT has also been reported in microwave optomechanical systems by Teufel *et al.* (32).

References and Notes

- M. Fleischhauer, A. Imamoglu, J. P. Marangos, *Rev. Mod. Phys.* **77**, 633 (2005).
- K.-J. Boller, A. Imamoglu, S. E. Harris, *Phys. Rev. Lett.* **66**, 2593 (1991).
- A. Kasapi, M. Jain, G. Y. Yin, S. E. Harris, *Phys. Rev. Lett.* **74**, 2447 (1995).
- L. V. Hau, S. E. Harris, Z. Dutton, C. H. Behroozi, *Nature* **397**, 594 (1999).
- D. F. Phillips, A. Fleischhauer, A. Mair, R. L. Walsworth, M. D. Lukin, *Phys. Rev. Lett.* **86**, 783 (2001).
- C. Liu, Z. Dutton, C. H. Behroozi, L. V. Hau, *Nature* **409**, 490 (2001).
- M. D. Lukin, A. Imamoglu, *Nature* **413**, 273 (2001).
- A. V. Turukhin *et al.*, *Phys. Rev. Lett.* **88**, 023602 (2002).
- D. Brunner *et al.*, *Science* **325**, 70 (2009).
- T. J. Kippenberg, H. Rokhsari, T. Carmon, A. Scherer, K. J. Vahala, *Phys. Rev. Lett.* **95**, 033901 (2005).
- A. Schliesser, P. Del'Haye, N. Nooshi, K. J. Vahala, T. J. Kippenberg, *Phys. Rev. Lett.* **97**, 243905 (2006).
- O. Arcizet, P.-F. Cohadon, T. Briant, M. Pinard, A. Heidmann, *Nature* **444**, 71 (2006).
- S. Gigan *et al.*, *Nature* **444**, 67 (2006).
- S. Gröblacher, K. Hammerer, M. R. Vanner, M. Aspelmeyer, *Nature* **460**, 724 (2009).
- Q. Lin *et al.*, *Nat. Photonics* **4**, 236 (2010).
- A. Schliesser, Cavity optomechanics and optical frequency comb generation with silica whispering-gallery-mode

microresonators, Thesis, Ludwig-Maximilians-Universität München (2009); <http://edoc.ub.uni-muenchen.de/10940>.

- A. Schliesser, T. J. Kippenberg, in *Advances in Atomic, Molecular and Optical Physics*, Vol. 58, E. Arimondo, P. Berman, C. C. Lin, Eds. (Elsevier Academic Press, 2010), pp. 207–323.
- G. S. Agarwal, S. Huang, *Phys. Rev. A* **81**, 041803 (2010).
- J. Zhang, K. Peng, S. L. Braunstein, *Phys. Rev. A* **68**, 013808 (2003).
- M. Mücke *et al.*, *Nature* **465**, 755 (2010).
- C. Fabre *et al.*, *Phys. Rev. A* **49**, 1337 (1994).
- F. Marquardt, J. P. Chen, A. A. Clerk, S. M. Girvin, *Phys. Rev. Lett.* **99**, 093902 (2007).
- J. M. Dobrindt, I. Wilson-Rae, T. J. Kippenberg, *Phys. Rev. Lett.* **101**, 263602 (2008).
- M. Cai, O. Painter, K. J. Vahala, *Phys. Rev. Lett.* **85**, 74 (2000).
- A. Schliesser, O. Arcizet, R. Rivière, G. Anetsberger, T. Kippenberg, *Nat. Phys.* **5**, 509 (2009).
- G. Anetsberger *et al.*, *Nat. Phys.* **5**, 909 (2009).
- C. A. Regal, J. D. Teufel, K. W. Lehnert, *Nat. Phys.* **4**, 555 (2008).
- M. Eichenfield, R. Camacho, J. Chan, K. J. Vahala, O. Painter, *Nature* **459**, 550 (2009).
- J. D. Thompson *et al.*, *Nature* **452**, 72 (2008).
- D. E. Chang, A. H. Safavi-Naeini, M. Hafezi, O. Painter, <http://arxiv.org/abs/1006.3829> (2010).
- M. Eichenfield, J. Chan, R. M. Camacho, K. J. Vahala, O. Painter, *Nature* **462**, 78 (2009).
- J. D. Teufel *et al.*; <http://arxiv.org/abs/1011.3067> (2010).
- T.J.K. acknowledges financial support by the Max Planck Society, European Research Council Starting Grant (SiMP), the European Union's MINOS, a Marie Curie Excellence Grant, the Swiss National Science Foundation, National Centre of Competence and Research of Quantum Photonics, and the Defense Advanced Research Projects Agency. S.D. is funded by a Marie Curie Individual Fellowship.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1195596/DC1
SOM Text

Figs. S1 to S3
References

26 July 2010; accepted 1 November 2010
Published online 11 November 2010;
10.1126/science.1195596

A Determination of the Cloud Feedback from Climate Variations over the Past Decade

A. E. Dessler

Estimates of Earth's climate sensitivity are uncertain, largely because of uncertainty in the long-term cloud feedback. I estimated the magnitude of the cloud feedback in response to short-term climate variations by analyzing the top-of-atmosphere radiation budget from March 2000 to February 2010. Over this period, the short-term cloud feedback had a magnitude of 0.54 ± 0.74 (2σ) watts per square meter per kelvin, meaning that it is likely positive. A small negative feedback is possible, but one large enough to cancel the climate's positive feedbacks is not supported by these observations. Both long- and short-wave components of short-term cloud feedback are also likely positive. Calculations of short-term cloud feedback in climate models yield a similar feedback. I find no correlation in the models between the short- and long-term cloud feedbacks.

Much of the global warming expected over the next century comes from feedbacks rather than direct warming from CO₂ and other greenhouse agents. Of these feedbacks,

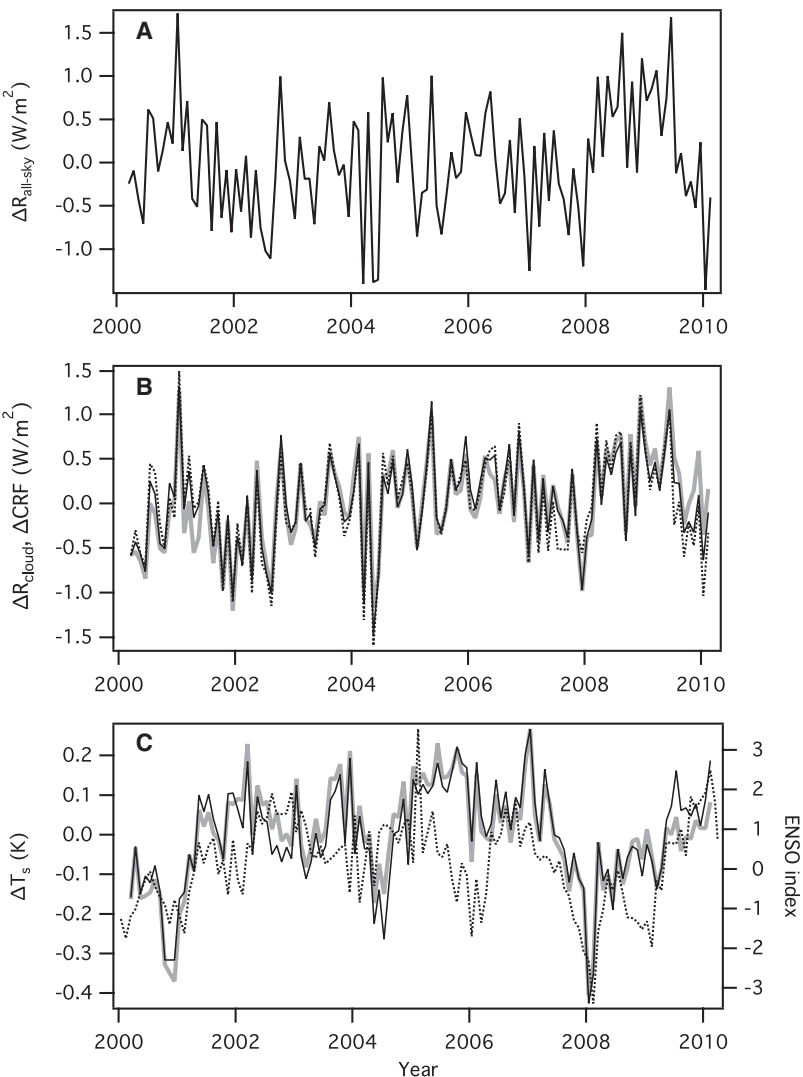
the most complex and least understood is the cloud feedback (1, 2). Clouds affect the climate by reflecting incoming solar radiation back to space, which tends to cool the climate, and by trapping

outgoing infrared radiation, which tends to warm the climate. In our present climate, the reflection of solar energy back to space dominates, and the net effect of clouds is to reduce the net flux of incoming energy at the top of the atmosphere (TOA) by ~ 20 W/m², as compared to an otherwise identical planet without clouds. The cloud feedback refers to changes in this net effect of clouds as the planet warms. If, as the climate warms, cloud changes further reduce net incoming energy, this will offset some of the warming, resulting in a negative cloud feedback. If, on the other hand, cloud changes lead to increases in net incoming energy, then the change will amplify the initial warming, resulting in a positive cloud feedback.

Climate models disagree on the magnitude of the cloud feedback, simulating a range of cloud feedbacks in response to long-term global warming from near zero to a positive feedback of 1 W/m²/K (3, 4). This spread is the single most important reason for the large spread in the climate

Department of Atmospheric Sciences, Texas A&M University, College Station, TX, USA. E-mail: adessler@tamu.edu

Fig. 1. (A) Global and monthly averaged $\Delta R_{\text{all-sky}}$, measured by CERES. (B) Global and monthly averaged ΔR_{cloud} calculated from CERES measurements and reanalyses (solid lines) and ΔCRF (dotted line) from the CERES and ECMWF interim reanalysis. (C) Global and monthly averaged ΔT_s from reanalyses (solid lines), along with an ENSO index (23) (dotted line). In (B) and (C), calculations using CERES data with the ECMWF interim reanalysis and MERRA are the solid black and gray lines, respectively. The sign convention used in this paper is that downward fluxes are positive.



sensitivities among climate models (5, 6). Despite the importance of the cloud feedback, there have been few estimates of its magnitude from observations. Previous work has generally focused on just part of the problem [for example, the tropics (7, 8) or low clouds (9)], and these analyses differ even on the sign of the cloud feedback.

In this paper, I present an estimate of the global cloud feedback in response to short-term climate fluctuations over the past decade and compare these results to those from climate models. The primary source of climate variations over this time period is the El Niño–Southern Oscillation (ENSO), which is a self-sustained coupled atmosphere–ocean mode of variability (10). During the El Niño phase, monthly and global-average surface temperature are several tenths of a degree Celsius warmer than during the La Niña phase, and these climate variations have previously been used to quantify the water vapor feedback (11, 12).

Figure 1A shows a time series of monthly and global-average anomalies of TOA net flux ($\Delta R_{\text{all-sky}}$) between March 2000 and February 2010 measured by the Clouds and the Earth’s Radiant Energy System (CERES) (13) instruments onboard NASA’s Terra satellite. This time series (14) is

Table 1. Cloud feedback values. All uncertainties are 2σ . Feedbacks are calculated from a 100-year segment of a control run, except for CCSM3, which is based on 80 years. N/A, not available.

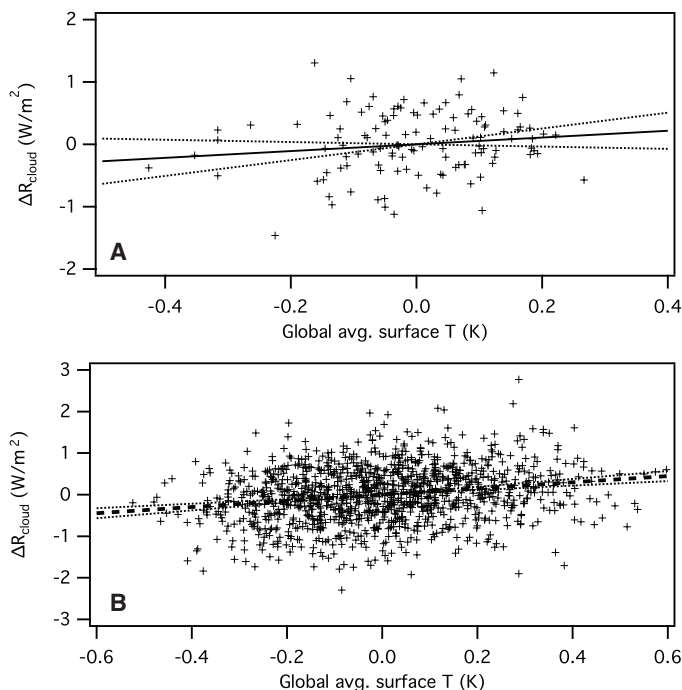
Model	Short-term cloud feedback			Long-term cloud feedback*	Equilibrium climate sensitivity†
	Total	Long-wave component	Short-wave component		
NCAR PCM1	1.11 ± 0.20	0.52 ± 0.11	0.60 ± 0.21	0.18	2.1
IPSL-CM4	1.05 ± 0.16	1.17 ± 0.13	-0.12 ± 0.14	1.06	4.4
INM-CM3.0	0.98 ± 0.18	0.77 ± 0.10	0.21 ± 0.19	0.35	2.1
UKMO-HadCM3	0.88 ± 0.31	0.57 ± 0.15	0.31 ± 0.35	1.08	3.3
ECHAM/MPI-OM	0.74 ± 0.20	0.97 ± 0.09	-0.23 ± 0.20	1.18	3.4
NCAR CCSM3	0.62 ± 0.26	0.17 ± 0.12	0.45 ± 0.25	0.14	2.7
GFDL-CM2.0	0.41 ± 0.22	-0.03 ± 0.11	0.43 ± 0.26	0.67	2.9
GFDL-CM2.1	0.34 ± 0.20	0.40 ± 0.08	-0.06 ± 0.23	0.81	3.4
ECMWF-CERES	0.54 ± 0.74	0.43 ± 0.45	0.12 ± 0.78	N/A	N/A
MERRA-CERES	0.46 ± 0.77	0.27 ± 0.47	0.19 ± 0.76	N/A	N/A

*From table 1 of Soden and Held (3). †Equilibrium climate change (in degrees kelvin) in response to a doubling of CO_2 [from table 8.2 of Randall *et al.* (1)].

stable to better than $0.5 \text{ W/m}^2/\text{decade}$ [the stability of the short-wave component is $0.3 \text{ W/m}^2/\text{decade}$ (15) and that of the long-wave component is $0.2 \text{ W/m}^2/\text{decade}$, from comparisons to Atmospheric Infrared Sounder measurements].

From these data, I extracted the part of $\Delta R_{\text{all-sky}}$ caused by changing clouds, hereafter referred to as ΔR_{cloud} . To do this, I took cloud radiative-forcing anomalies (ΔCRF) and adjusted those to account for the impact of changing tem-

Fig. 2. (A) Scatter plot of monthly average values of ΔR_{cloud} versus ΔT_s using CERES and ECMWF interim data. **(B)** Scatter plot of monthly averages of the same quantities from 100 years of a control run of the ECHAM/MPI-OM model. In all plots, the solid line is a linear least-squares fit and the dotted lines are the 2σ confidence interval of the fit.



perature, water vapor, surface albedo, and radiative forcing, ultimately yielding ΔR_{cloud} (16, 17). ΔCRF is the change in TOA net flux anomaly if clouds were instantaneously removed, with everything else held fixed, and it is determined by subtracting $\Delta R_{\text{all-sky}}$, obtained from CERES measurements, from the clear-sky flux anomalies $\Delta R_{\text{clear-sky}}$, obtained from a reanalysis system. The ΔCRF time series is plotted in Fig. 1B.

In a reanalysis system, conventional and satellite-based meteorological observations are combined within a weather forecast assimilation system in order to produce a global and physically consistent picture of the state of the atmosphere. I used both the ECMWF (European Centre for Medium-Range Weather Forecasts) interim reanalysis (18) and NASA's Modern Era Retrospective analysis for Research and Applications (MERRA) (19) in the calculations. The fields being used here (mainly water vapor and temperature) are constrained in the reanalysis by high-density satellite measurements. Previous work has shown that $\Delta R_{\text{clear-sky}}$ can be calculated accurately given water vapor and temperature distributions (20, 21). And, given suggestions of biases in measured clear-sky fluxes (22), I chose to use the reanalysis fluxes here.

The water vapor, temperature, and surface albedo anomalies used to convert ΔCRF into ΔR_{cloud} also come from the reanalyses. Additionally, the all-sky radiative forcing change caused primarily by changes in long-lived greenhouse gases over the March 2000–February 2010 period is estimated to be $+0.25 \text{ W/m}^2$. Following Soden *et al.* (16), I multiply the all-sky radiative forcing by 0.16 to estimate the difference between clear-sky and all-sky radiative forcing. Anomalies of all quantities are calculated by subtracting from each month's value the average for that month over the entire time series. Figure 1B

shows time series of ΔR_{cloud} , and it shows that the differences between ΔCRF and ΔR_{cloud} are small for these data. For compactness, I will refer to the calculated values of ΔR_{cloud} as “the observations.”

Figure 1C shows the accompanying time series of global-average and monthly mean surface temperature anomalies (ΔT_s) from the reanalyses. Also plotted is an ENSO index (23), and the close association between that and ΔT_s verifies that ENSO is the primary source of variations in ΔT_s .

The cloud feedback is conventionally defined as the change in ΔR_{cloud} per unit of change in ΔT_s . Figure 2A is a scatter plot of monthly values of ΔR_{cloud} versus ΔT_s , calculated using ECMWF interim meteorological fields. The slope of this scatter plot is the strength of the cloud feedback, and it is estimated by a traditional least-squares fit to be $0.54 \pm 0.72 \text{ (} 2\sigma \text{) W/m}^2/\text{K}$ (the slope using the MERRA is $0.46 \pm 0.75 \text{ W/m}^2/\text{K}$). Because I have defined downward flux as positive, the positive slope here means that, as the surface warms, clouds trap additional energy; in other words, the cloud feedback here is positive.

The uncertainty quoted above is the statistical uncertainty of the fit. The impact of a spurious long-term trend in either $\Delta R_{\text{all-sky}}$ or $\Delta R_{\text{clear-sky}}$ is estimated by adding in a trend of $\pm 0.5 \text{ W/m}^2/\text{decade}$ into the CERES data. This changes the calculated feedback by $\pm 0.18 \text{ W/m}^2/\text{K}$. Adding these errors in quadrature yields a total uncertainty of 0.74 and 0.77 $\text{W/m}^2/\text{K}$ in the calculations, using the ECMWF and MERRA reanalyses, respectively. Other sources of uncertainty are negligible.

Given the uncertainty, the possibility of a small negative feedback cannot be excluded. There have been inferences (7, 8) of a large negative cloud feedback in response to short-term climate variations that can substantially cancel the other

feedbacks operating in our climate system. This would require the cloud feedback to be in the range of -1.0 to $-1.5 \text{ W/m}^2/\text{K}$ or larger, and I see no evidence to support such a large negative cloud feedback [these inferences of large negative feedbacks have also been criticized on methodological grounds (24, 25)].

I have not explicitly considered the direct effect of aerosols on TOA flux in this analysis. The effects of aerosols are obviously included in the $\Delta R_{\text{all-sky}}$ measurements, but the $\Delta R_{\text{clear-sky}}$ calculations include only an annual-cycle climatology. As a result, the radiative impact of interannual variations in aerosols will be included in ΔR_{cloud} . But aerosols' radiative impact is not expected to correlate with ΔT_s , so the effect of aerosols is to add uncertainty to the cloud feedback calculation but should not introduce a bias.

This definition of the cloud feedback is a standard approach for quantifying feedbacks (26). It only requires an association between T_s and ΔR_{cloud} but does not imply any specific physical mechanism connecting them. The recent suggestion that feedback analyses suffer from a cause-and-effect problem (27) does not apply here: The climate variations being analyzed here are primarily driven by ENSO, and there has been no suggestion that ENSO is caused by cloud variations (10).

Obviously, the correlation between ΔR_{cloud} and ΔT_s is weak ($r^2 = 2\%$), meaning that factors other than T_s are important in regulating ΔR_{cloud} . An example is the Madden-Julian Oscillation (7), which has a strong impact on ΔR_{cloud} but no effect on ΔT_s . This does not mean that ΔT_s exerts no control on ΔR_{cloud} , but rather that the influence is hard to quantify because of the influence of other factors. As a result, it may require several more decades of data to significantly reduce the uncertainty in the inferred relationship.

In this way, the cloud feedback is quite different from the water vapor feedback. The water vapor feedback is primarily controlled by tropical upper-tropospheric water vapor (28), which in turn is strongly controlled by tropical surface temperatures (11, 29). Because of this, the relationship between surface temperature and the radiative impact of water vapor is tight and completely clear in just a few years of data. This is one of the main reasons why our confidence in the water vapor feedback is so strong (30).

One obvious question is whether climate models also reproduce this cloud feedback in response to short-term climate variations. To test this, I analyzed control runs from fully coupled climate models, in which atmospheric greenhouse gas abundances and other forcings are held constant at either their preindustrial or present-day concentrations; thus, there are no long-term trends in the models' climate, and the climate variations in the model runs are entirely due to internal variability. The control runs were obtained from the World Climate Research Programme's (WCRP's) Coupled Model Intercomparison Project phase 3 (CMIP3) multimodel data set (31).

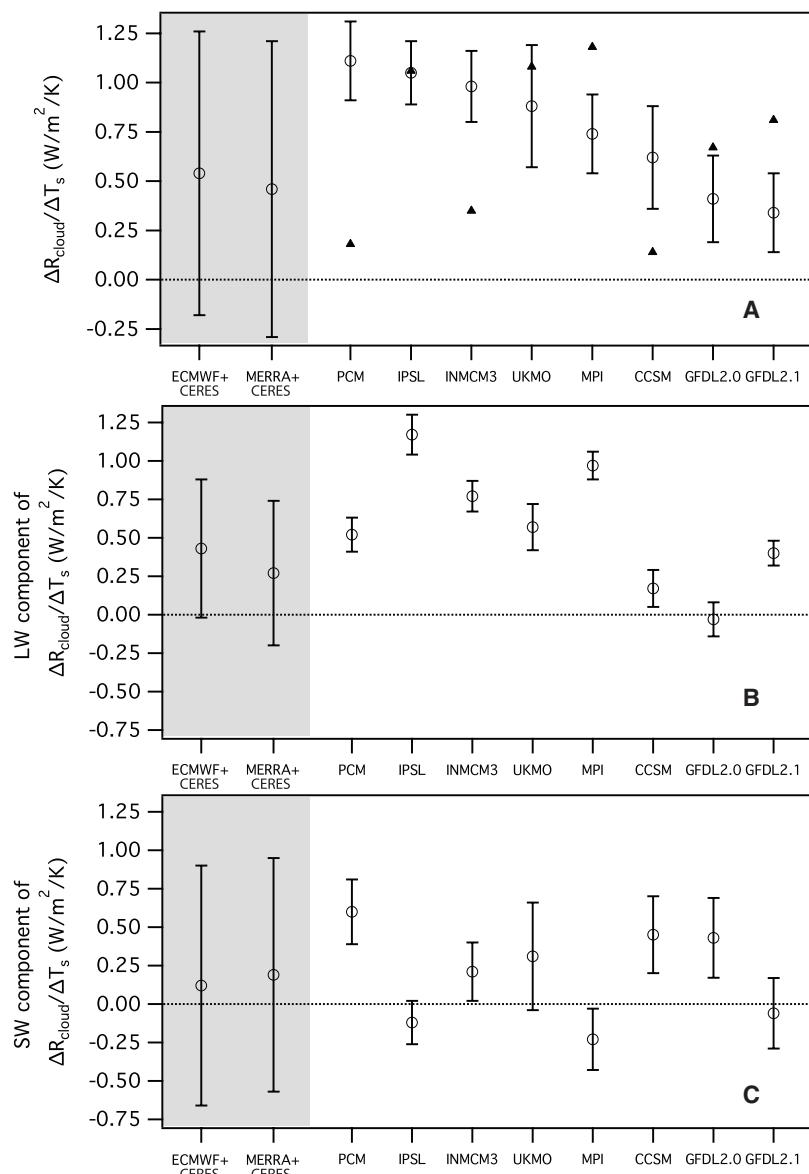


Fig. 3. (A) Comparison of the total cloud feedback in response to short-term climate variations (circles with error bars); triangles represent the cloud feedback in response to long-term warming [from table 1 of Soden and Held (3)]. (B) Comparison of the long-wave component of the cloud feedback in response to short-term climate variations. (C) Comparison of the short-wave component of the cloud feedback in response to short-term climate variations.

Figure 2B shows a scatter plot of ΔR_{cloud} versus ΔT_s from 100 years of a control run of the ECHAM/MPI-OM climate model, obtained using exactly the same method as was used to analyze the observations. The cloud feedback in the model in response to short-term climate variations is $0.74 \pm 0.20 \text{ W/m}^2/\text{K}$, which is in reasonable agreement with the observations. r^2 for the fit is about 4%, showing that the models also reproduce the relatively weak control exerted by ΔT_s on ΔR_{cloud} . Table 1 lists the cloud feedback in response to short-term climate variations for eight climate models; these values are also plotted in Fig. 3A.

The models' cloud feedbacks range from 0.34 ± 0.20 to $1.11 \pm 0.20 \text{ W/m}^2/\text{K}$. Thus, the models paint a consistent picture of positive cloud feedbacks in response to short-term climate variations.

The observations fall within the range of models, and taken as a group, there is substantial agreement between the observations and the models' cloud feedback. However, given the large uncertainties, the observations are currently of no obvious help in determining which models most accurately simulate the cloud feedback.

Table 1 and Fig. 3A also show the cloud feedback in response to long-term (centennial-scale) climate change, and there is no correlation between the short-term and long-term cloud feedbacks. This means that even if some models could be excluded on the basis of their short-term climate feedback, it would not necessarily get us any closer to reducing the range of equilibrium climate sensitivity because of the apparent time-scale dependence of the cloud feedback. It should be noted that, be-

cause of correlations between the feedbacks (32), this analysis does not preclude the possibility that short-term radiative damping rates might still correlate with equilibrium climate sensitivity (33).

The long- and short-wave components of the cloud feedbacks are also listed in Table 1 and plotted in Fig. 3, B and C. The observations show that 60 to 80% of the total cloud feedback comes from a positive long-wave feedback, with the rest coming from a weaker and highly uncertain positive short-wave feedback. With the exception of one model, the models also produce positive long-wave cloud feedbacks, a result also in accord with simple theoretical arguments (34).

The sign of the short-wave feedback shows more variation among models; it is positive in five of the models and negative in three. There is also a clear tendency for models to compensate for the strength of one feedback with weakness in another. The models with the strongest short-wave feedbacks tend to have the weakest long-wave feedbacks, whereas models with the weakest short-wave feedbacks have the strongest long-wave feedbacks.

Finally, both observations and models have smaller uncertainties in the long-wave feedback than in the short-wave feedback. This means that the long-wave component of ΔR_{cloud} correlates more closely with ΔT_s than the short-wave component.

For the problem of long-term climate change, what we really want to determine is the cloud feedback in response to long-term climate change. Unfortunately, it may be decades before a direct measurement is possible. In the meantime, observing shorter-term climate variations and comparing those observations to climate models may be the best we can do. This is what I have done in this paper. My analysis suggests that the short-term cloud feedback is likely positive and that climate models as a group are doing a reasonable job of simulating this feedback, providing some indication that models successfully simulate the response of clouds to climate variations. However, owing to the apparent time-scale dependence of the cloud feedback and the uncertainty in the observed short-term cloud feedback, we cannot use this analysis to reduce the present range of equilibrium climate sensitivity of 2.0 to 4.5 K.

References and Notes

1. D. A. Randall et al., in *Climate Change 2007: The Physical Science Basis. Contributions of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, S. Solomon et al., Eds. (Cambridge Univ. Press, Cambridge, 2007).
2. G. L. Stephens, *J. Clim.* **18**, 237 (2005).
3. B. J. Soden, I. M. Held, *J. Clim.* **19**, 3354 (2006).
4. R. Colman, *Clim. Dyn.* **20**, 865 (2003).
5. M. J. Webb et al., *Clim. Dyn.* **27**, 17 (2006).
6. J. L. Dufresne, S. Bony, *J. Clim.* **21**, 5135 (2008).
7. R. W. Spencer, W. D. Braswell, J. R. Christy, J. Hnilo, *Geophys. Res. Lett.* **34**, L15707 (2007).
8. R. S. Lindzen, Y. S. Choi, *Geophys. Res. Lett.* **36**, L16705 (2009).
9. A. C. Clement, R. Burgman, J. R. Norris, *Science* **325**, 460 (2009).
10. J. D. Neelin et al., *J. Geophys. Res.* **103**, 14261 (1998).
11. A. E. Dessler, S. Wong, *J. Clim.* **22**, 6404 (2009).

12. A. E. Dessler, P. Yang, Z. Zhang, *Geophys. Res. Lett.* **35**, L20704 (2008).
13. B. A. Wielicki et al., *Bull. Am. Meteorol. Assoc.* **77**, 853 (1996).
14. The data used here are the SSF Edition 2.5 $1^\circ \times 1^\circ$ gridded, monthly averaged data. Edition 2.5 contains new Edition 3 calibration combined with Edition 2 algorithms and is available from <http://ceres.larc.nasa.gov>.
15. N. G. Loeb et al., *J. Clim.* **20**, 575 (2007).
16. B. J. Soden et al., *J. Clim.* **21**, 3504 (2008).
17. K. M. Shell, J. T. Kiehl, C. A. Shields, *J. Clim.* **21**, 2269 (2008).
18. A. Simmons, S. Uppala, D. Dee, S. Kobayashi, ERA-Interim: New ECMWF reanalysis products from 1989 onward (*ECMWF Newsletter* **110**, Winter 2006/2007). Data can be downloaded from www.ecmwf.int/products/data.
19. M. J. Suarez et al., The GEOS-5 Data Assimilation System—documentation of versions 5.0.1, 5.1.0, and 5.2.0, NASA/TM–2008–104606, Vol. 27, 2008. Data can be downloaded from <http://disc.sci.gsfc.nasa.gov>.
20. A. E. Dessler et al., *J. Geophys. Res.* **113**, D17102 (2008).
21. L. Moy et al., *J. Geophys. Res.* **115**, D15110 (2010).
22. B. J. Soden, R. Bennart, *J. Geophys. Res.* **113**, D20107 (2008).
23. C. A. Smith, P. D. Sardeshmukh, *Int. J. Climatol.* **20**, 1543 (2000).
24. K. E. Trenberth, J. T. Fasullo, C. O'Dell, T. Wong, *Geophys. Res. Lett.* **37**, L03702 (2010).
25. D. M. Murphy, *Geophys. Res. Lett.* **37**, L09704 (2010).
26. M. H. Zhang, J. J. Hack, J. T. Kiehl, R. D. Cess, *J. Geophys. Res.* **99**, 5525 (1994).
27. R. W. Spencer, W. D. Braswell, *J. Geophys. Res.* **115**, D16109 (2010).
28. I. M. Held, B. J. Soden, *Annu. Rev. Energy Environ.* **25**, 441 (2000).
29. K. Minschwaner, A. E. Dessler, *J. Clim.* **17**, 1272 (2004).
30. A. E. Dessler, S. C. Sherwood, *Science* **323**, 1020 (2009).
31. G. A. Meehl et al., *Bull. Am. Meteorol. Soc.* **88**, 1383 (2007).
32. P. Huybers, *J. Clim.* **23**, 3009 (2010).
33. E. S. Chung, B. J. Soden, B. J. Sohn, *Geophys. Res. Lett.* **37**, L10703 (2010).
34. M. Zelinka, D. L. Hartmann, *J. Geophys. Res.* **115**, D16117 (2010).
35. I thank the CERES, MERRA, and ECMWF groups for producing the data used in this paper. Support for this work came from NASA grant NNX08AR27G to Texas A&M University. I also acknowledge useful contributions from N. Loeb, M. Bosilovich, J.-J. Morcrette, M. Zelinka, P. Stackhouse, T. Wong, J. Fasullo, P. Yang, and G. North. Finally, I acknowledge the modeling groups, the Program for Climate Model Diagnosis and Intercomparison, and the WCRP's Working Group on Coupled Modeling for their roles in making available the WCRP CMIP3 multimodel data set. Support of this data set is provided by the U.S. Department of Energy Office of Science.

19 May 2010; accepted 9 November 2010
 10.1126/science.1192546

Stochastic Late Accretion to Earth, the Moon, and Mars

William F. Bottke,^{1*} Richard J. Walker,² James M. D. Day,^{2,3}
 David Nesvorný,¹ Linda Elkins-Tanton⁴

Core formation should have stripped the terrestrial, lunar, and martian mantles of highly siderophile elements (HSEs). Instead, each world has disparate, yet elevated HSE abundances. Late accretion may offer a solution, provided that $\geq 0.5\%$ Earth masses of broadly chondritic planetesimals reach Earth's mantle and that ~ 10 and ~ 1200 times less mass goes to Mars and the Moon, respectively. We show that leftover planetesimal populations dominated by massive projectiles can explain these additions, with our inferred size distribution matching those derived from the inner asteroid belt, ancient martian impact basins, and planetary accretion models. The largest late terrestrial impactors, at 2500 to 3000 kilometers in diameter, potentially modified Earth's obliquity by $\sim 10^\circ$, whereas those for the Moon, at ~ 250 to 300 kilometers, may have delivered water to its mantle.

Highly siderophile elements (HSEs: Re, Os, Ir, Ru, Pt, Rh, Pd, Au) have low-pressure metal-silicate partition coefficients that are extremely high ($>10^4$) (1). Hence, a common assumption has been that the silicate portions of rocky planetary bodies with metallic cores are effectively stripped of HSEs immediately after primary accretion and final core segregation (2). Accordingly, the “giant impact” on Earth that formed the Moon 60^{+90}_{-10} million years (My) after formation of the earliest solids should have cleansed HSEs from the mantles of both worlds (3–5).

However, studies of mantle-derived terrestrial peridotites (olivine-rich rocks that dominate Earth's upper mantle) have shown that, not only are HSE abundances in Earth's mantle much higher than expected (at $\sim 0.008 \times \text{CI-chondrite}$

meteorites), but their HSE proportions are also approximately the same as chondritic meteorites (Fig. 1) (6). Although we have no direct samples of martian or lunar mantle rocks, studies of HSE and Os isotopes in derivative mantle melts sug-

gest roughly equivalent absolute abundances in the martian mantle (7, 8), but much lower abundances in the lunar mantle ($\leq 0.0004 \times \text{CI-chondrite}$) (9–11), with HSEs in chondritic relative proportions for both bodies (Fig. 1) (7, 10).

Although different scenarios have been proposed to produce the relatively high absolute and chondritic relative abundances of HSEs in planetary mantles (12), perhaps the most straightforward process is delivery from continued planetesimal accretion after the last core-formation event, with the materials mixed into the mantle by convection (13). Such events would represent a natural continuum from a planet-formation perspective, with the Mars-sized projectile that produced the giant impact representing the largest component of the leftover planetesimal population that continued to bombard the planets until surviving projectiles were depleted by collisional and dynamical processes (14).

We used the estimated collective distribution of HSEs in the terrestrial, martian, and lunar mantles to test whether their abundances were set by late accretion. For Earth, late accretion of $\sim 2.0 \times 10^{22}$ kg of material with bulk chondritic

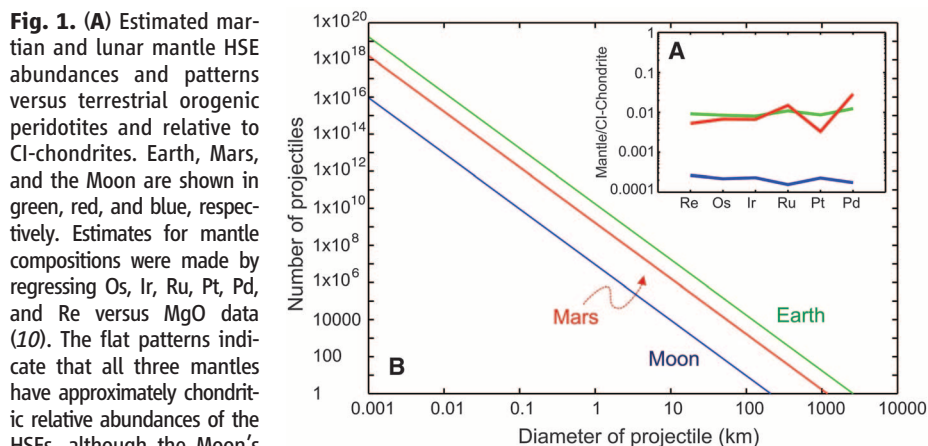


Fig. 1. (A) Estimated martian and lunar mantle HSE abundances and patterns versus terrestrial orogenic peridotites and relative to CI-chondrites. Earth, Mars, and the Moon are shown in green, red, and blue, respectively. Estimates for mantle compositions were made by regressing Os, Ir, Ru, Pt, Pd, and Re versus MgO data (10). The flat patterns indicate that all three mantles have approximately chondritic relative abundances of the HSEs, although the Moon's mantle abundances are >20 times less than those of Earth. **(B)** Minimum number and sizes of late accretion chondritic projectiles needed to deliver the estimated abundances of HSEs to the mantles of the Moon, Mars, and Earth, assuming 100% accretion efficiency. These values are lower limits in terms of delivered mass because the process is unlikely to be 100% efficient.

¹Southwest Research Institute and NASA Lunar Science Institute, 1050 Walnut Street, Suite 400, Boulder, CO 80302, USA. ²Department of Geology, University of Maryland, College Park, MD 20742, USA. ³Geosciences Research Division, Scripps Institution of Oceanography, La Jolla, CA 92093, USA. ⁴Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

*To whom correspondence should be addressed. E-mail: bottke@boulder.swri.edu

12. A. E. Dessler, P. Yang, Z. Zhang, *Geophys. Res. Lett.* **35**, L20704 (2008).
13. B. A. Wielicki et al., *Bull. Am. Meteorol. Assoc.* **77**, 853 (1996).
14. The data used here are the SSF Edition 2.5 $1^\circ \times 1^\circ$ gridded, monthly averaged data. Edition 2.5 contains new Edition 3 calibration combined with Edition 2 algorithms and is available from <http://ceres.larc.nasa.gov>.
15. N. G. Loeb et al., *J. Clim.* **20**, 575 (2007).
16. B. J. Soden et al., *J. Clim.* **21**, 3504 (2008).
17. K. M. Shell, J. T. Kiehl, C. A. Shields, *J. Clim.* **21**, 2269 (2008).
18. A. Simmons, S. Uppala, D. Dee, S. Kobayashi, ERA-Interim: New ECMWF reanalysis products from 1989 onward (*ECMWF Newsletter* **110**, Winter 2006/2007). Data can be downloaded from www.ecmwf.int/products/data.
19. M. J. Suarez et al., The GEOS-5 Data Assimilation System—documentation of versions 5.0.1, 5.1.0, and 5.2.0, NASA/TM–2008–104606, Vol. 27, 2008. Data can be downloaded from <http://disc.sci.gsfc.nasa.gov>.
20. A. E. Dessler et al., *J. Geophys. Res.* **113**, D17102 (2008).
21. L. Moy et al., *J. Geophys. Res.* **115**, D15110 (2010).
22. B. J. Soden, R. Bennart, *J. Geophys. Res.* **113**, D20107 (2008).
23. C. A. Smith, P. D. Sardeshmukh, *Int. J. Climatol.* **20**, 1543 (2000).
24. K. E. Trenberth, J. T. Fasullo, C. O'Dell, T. Wong, *Geophys. Res. Lett.* **37**, L03702 (2010).
25. D. M. Murphy, *Geophys. Res. Lett.* **37**, L09704 (2010).
26. M. H. Zhang, J. J. Hack, J. T. Kiehl, R. D. Cess, *J. Geophys. Res.* **99**, 5525 (1994).
27. R. W. Spencer, W. D. Braswell, *J. Geophys. Res.* **115**, D16109 (2010).
28. I. M. Held, B. J. Soden, *Annu. Rev. Energy Environ.* **25**, 441 (2000).
29. K. Minschwaner, A. E. Dessler, *J. Clim.* **17**, 1272 (2004).
30. A. E. Dessler, S. C. Sherwood, *Science* **323**, 1020 (2009).
31. G. A. Meehl et al., *Bull. Am. Meteorol. Soc.* **88**, 1383 (2007).
32. P. Huybers, *J. Clim.* **23**, 3009 (2010).
33. E. S. Chung, B. J. Soden, B. J. Sohn, *Geophys. Res. Lett.* **37**, L10703 (2010).
34. M. Zelinka, D. L. Hartmann, *J. Geophys. Res.* **115**, D16117 (2010).
35. I thank the CERES, MERRA, and ECMWF groups for producing the data used in this paper. Support for this work came from NASA grant NNX08AR27G to Texas A&M University. I also acknowledge useful contributions from N. Loeb, M. Bosilovich, J.-J. Morcrette, M. Zelinka, P. Stackhouse, T. Wong, J. Fasullo, P. Yang, and G. North. Finally, I acknowledge the modeling groups, the Program for Climate Model Diagnosis and Intercomparison, and the WCRP's Working Group on Coupled Modeling for their roles in making available the WCRP CMIP3 multimodel data set. Support of this data set is provided by the U.S. Department of Energy Office of Science.

19 May 2010; accepted 9 November 2010
 10.1126/science.1192546

Stochastic Late Accretion to Earth, the Moon, and Mars

William F. Bottke,^{1*} Richard J. Walker,² James M. D. Day,^{2,3}
 David Nesvorný,¹ Linda Elkins-Tanton⁴

Core formation should have stripped the terrestrial, lunar, and martian mantles of highly siderophile elements (HSEs). Instead, each world has disparate, yet elevated HSE abundances. Late accretion may offer a solution, provided that $\geq 0.5\%$ Earth masses of broadly chondritic planetesimals reach Earth's mantle and that ~ 10 and ~ 1200 times less mass goes to Mars and the Moon, respectively. We show that leftover planetesimal populations dominated by massive projectiles can explain these additions, with our inferred size distribution matching those derived from the inner asteroid belt, ancient martian impact basins, and planetary accretion models. The largest late terrestrial impactors, at 2500 to 3000 kilometers in diameter, potentially modified Earth's obliquity by $\sim 10^\circ$, whereas those for the Moon, at ~ 250 to 300 kilometers, may have delivered water to its mantle.

Highly siderophile elements (HSEs: Re, Os, Ir, Ru, Pt, Rh, Pd, Au) have low-pressure metal-silicate partition coefficients that are extremely high ($>10^4$) (1). Hence, a common assumption has been that the silicate portions of rocky planetary bodies with metallic cores are effectively stripped of HSEs immediately after primary accretion and final core segregation (2). Accordingly, the “giant impact” on Earth that formed the Moon 60^{+90}_{-10} million years (My) after formation of the earliest solids should have cleansed HSEs from the mantles of both worlds (3–5).

However, studies of mantle-derived terrestrial peridotites (olivine-rich rocks that dominate Earth's upper mantle) have shown that, not only are HSE abundances in Earth's mantle much higher than expected (at $\sim 0.008 \times \text{CI-chondrite}$

meteorites), but their HSE proportions are also approximately the same as chondritic meteorites (Fig. 1) (6). Although we have no direct samples of martian or lunar mantle rocks, studies of HSE and Os isotopes in derivative mantle melts sug-

gest roughly equivalent absolute abundances in the martian mantle (7, 8), but much lower abundances in the lunar mantle ($\leq 0.0004 \times \text{CI-chondrite}$) (9–11), with HSEs in chondritic relative proportions for both bodies (Fig. 1) (7, 10).

Although different scenarios have been proposed to produce the relatively high absolute and chondritic relative abundances of HSEs in planetary mantles (12), perhaps the most straightforward process is delivery from continued planetesimal accretion after the last core-formation event, with the materials mixed into the mantle by convection (13). Such events would represent a natural continuum from a planet-formation perspective, with the Mars-sized projectile that produced the giant impact representing the largest component of the leftover planetesimal population that continued to bombard the planets until surviving projectiles were depleted by collisional and dynamical processes (14).

We used the estimated collective distribution of HSEs in the terrestrial, martian, and lunar mantles to test whether their abundances were set by late accretion. For Earth, late accretion of $\sim 2.0 \times 10^{22}$ kg of material with bulk chondritic

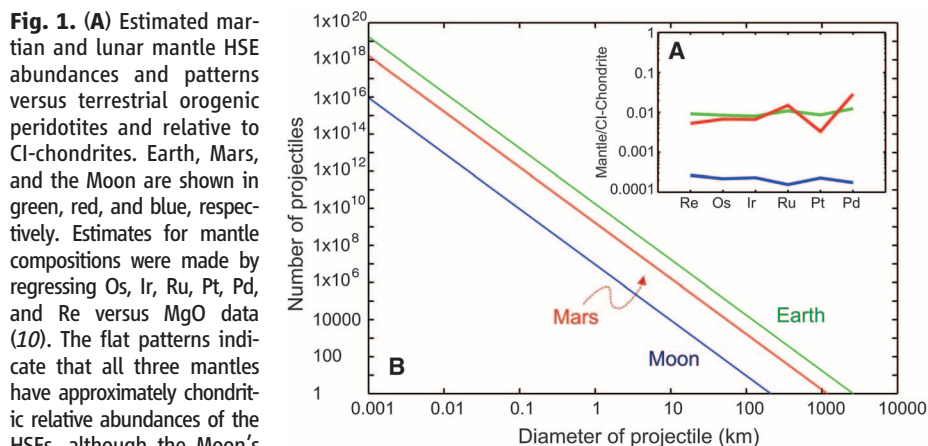


Fig. 1. (A) Estimated martian and lunar mantle HSE abundances and patterns versus terrestrial orogenic peridotites and relative to CI-chondrites. Earth, Mars, and the Moon are shown in green, red, and blue, respectively. Estimates for mantle compositions were made by regressing Os, Ir, Ru, Pt, Pd, and Re versus MgO data (10). The flat patterns indicate that all three mantles have approximately chondritic relative abundances of the HSEs, although the Moon's mantle abundances are >20 times less than those of Earth. **(B)** Minimum number and sizes of late accretion chondritic projectiles needed to deliver the estimated abundances of HSEs to the mantles of the Moon, Mars, and Earth, assuming 100% accretion efficiency. These values are lower limits in terms of delivered mass because the process is unlikely to be 100% efficient.

¹Southwest Research Institute and NASA Lunar Science Institute, 1050 Walnut Street, Suite 400, Boulder, CO 80302, USA. ²Department of Geology, University of Maryland, College Park, MD 20742, USA. ³Geosciences Research Division, Scripps Institution of Oceanography, La Jolla, CA 92093, USA. ⁴Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

*To whom correspondence should be addressed. E-mail: bottke@boulder.swri.edu

composition is required to reproduce measured mantle peridotite compositions (15), assuming even distribution of the HSEs throughout the mantle (12). This quantity of mass does not violate Earth-Moon isotopic constraints, such as for oxygen, examined to date (16, 17). If martian mantle abundances of the HSEs are similar to the terrestrial mantle (we assume that HSE abundances are $0.7 \times$ terrestrial mantle abundances), late accretion of $\sim 2.0 \times 10^{21}$ kg of material would be necessary for that planet (15). A proportionally much lower amount of mass ($\sim 1.7 \times 10^{19}$ kg) is needed for the Moon (10, 15), assuming that lunar mantle abundances are $\leq 0.05 \times$ terrestrial mantle abundances and the lunar core is only $\sim 4\%$ of the Moon's mass (11). These mass estimates yield Earth/Mars and Earth/Moon input mass ratios of ~ 10 and ~ 1200 , respectively.

The high Earth/Moon input mass ratio is curious on several levels. For example, the Earth/Moon impact number flux ratio for both late-accreting planetesimals and present-day near-Earth objects is ~ 20 , with this value being a reflection of their different gravitational cross-sectional areas (14, 18, 19). Micrometeorites, many of which achieve nearly circular orbits with Earth and the Moon via nongravitational forces

before impact, only reach an impact number flux value of ~ 50 , with the increase coming from gravitational focusing by Earth (19). Thus, it seems unlikely that small, numerous projectiles could ever achieve mass input ratios close to 1200 in the aftermath of the Moon-forming event when most leftover planetesimals, asteroids, or comets in the inner solar system were dynamically excited (14).

It is possible to lower the Earth/Moon input mass ratio by assuming that the Moon retained less impactor material than did Earth or Mars. Numerical simulations show that ~ 60 and $\sim 15\%$ of the mass of stony and water-ice impactors residing in the inner solar system, respectively, are retained on the Moon after an impact (20). Water-ice cannot carry HSEs, so a reasonable retention factor for standard cometary ice-rock compositions is $\sim 30\%$ (21). This could conceivably lower the ratio to between 400 and 700. However, if projectiles delivering HSEs were denser and/or volatile-poor, such as differentiated or iron-rich planetesimals, more mass might be retained, moving the range to 700 to 1200, or higher.

An additional constraint is that most HSEs had to be delivered to the lunar, terrestrial, and martian mantles within tens of million years of

core-formation termination. The lunar crust, which formed 4.42 to 4.46 billion years ago (Ga) or possibly earlier, is essentially intact and has only been modestly contaminated by extra-lunar materials (5, 10–12, 22). Thus, most of the HSEs in the terrestrial and lunar mantles arrived before this time. Late-arriving projectiles could potentially modify Earth's HSE budget, because their mass could reach the mantle via plate tectonic processes, but the net mass delivered would be limited because some fraction would unavoidably end up on the Moon where it is not observed. For Mars, Nd-Os isotopic correlations indicate that mantle reservoirs were isolated within ~ 20 My of differentiation (7). This implies that most HSEs were delivered by leftover debris from terrestrial planet accretion, which was probably dominated by stony, iron, and/or differentiated planetesimals, rather than comets (6, 23). Accordingly, the Earth/Moon input mass ratio is probably > 700 .

By combining these constraints within a Monte Carlo code, we explored whether any plausible late accretion size distributions were capable of delivering the estimated abundances of mass and, consequently, HSEs to Earth, Mars, and the Moon (24). For Earth and the Moon, whose origins and last core-formation events are linked together in time, and assuming a projectile size distribution $dN \propto D^{-q}dD$ (where dN is the number of objects of diameter D within a bin dD , and q is the differential power-law index), our best results came from simulations where $q < 2$ and few projectiles between 200 and 4000 km diameter hit the Moon (Fig. 2). This combination allowed large impactors to produce profound differences in delivered mass and HSEs to the mantles of the worlds they struck.

Although the precise projectile size range needed to match Earth/Moon constraints is unknown, modeling work does set limits (16). In the absence of plate tectonics, late accretion impactors needed to be large enough to breach early planetary lithospheres, create local magma ponds from their impact energy, and then efficiently mix into the mantle, but not so large that their impact-fragmented core coalesced with the planet's core. Assuming an end-member case (25), where a world is still in a magma ocean phase, the iron core of a differentiated projectile, assumed here to be about half the projectile's diameter, will become emulsified into the mantle if it is smaller than the depth of the magma ocean. For Earth, Mars, and the Moon, this criterion roughly limits HSE delivery among differentiated projectiles to diameters < 2000 to 4000 km (26), < 1000 to 3000 km (27), and < 1000 km (5), respectively. Projectiles striking after this phase need to punch through the body's newly formed lithosphere, and some will not make it. For those that do, fragmentation experienced en route to the mantle may break down the core into smaller pieces more suitable for emulsification (16).

For projectiles larger than 10% of the diameter of the target world, HSE delivery is mainly

Fig. 2. Results from a Monte Carlo simulation showing the differential power-law index q of the SFDs most likely to deliver the appropriate quantity of late accretion projectiles to Earth and the Moon (24). The colored lines correspond to the maximum projectile size that could potentially strike the worlds ($D_{\max}$). Low q values correspond to shallow size distributions where most of the mass of the colliding population is in the largest projectiles.

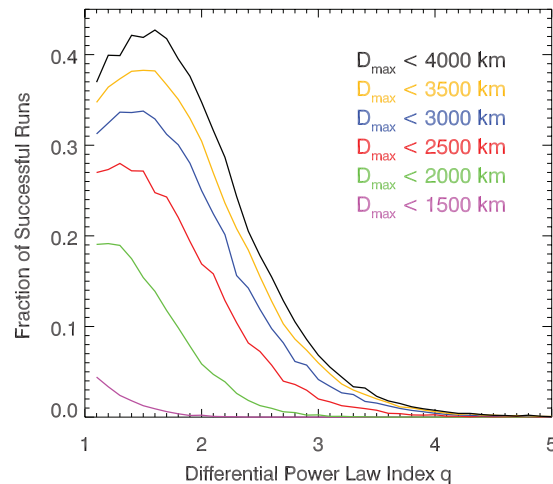
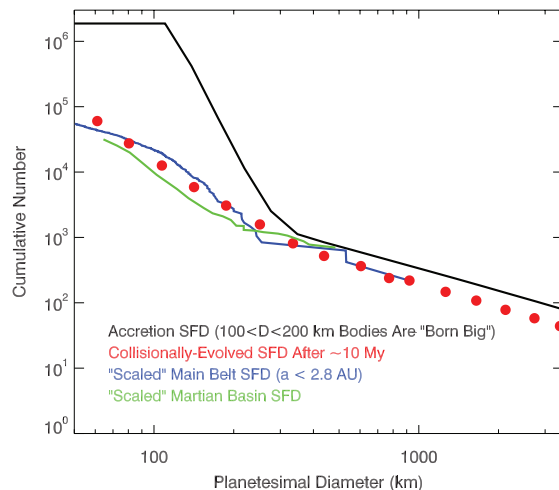


Fig. 3. SFDs of planetesimal populations at different evolution stages compared with data from the asteroid belt and ancient martian basins. The black curve represents an accretion SFD at 1 AU with planetesimals born big between $D \sim 100$ and 200 km. The shallow branch of the SFD for $D > 250$ km was produced by runaway growth and has $q \sim 2.2$. The red dots show that ~ 10 My of collisional evolution among the accretion SFD does not affect the slope of the $D > 250$ -km objects. The blue curve shows a scaled version of the inner and central main belt SFDs, defined as asteroids with semimajor axis $a < 2.8$ AU. The green line shows the estimated projectile SFD computed from martian impact basins.



controlled by the physics of so-called “hit and almost run” collisions (28), in which most of the projectile’s core (and HSEs) plows through the target’s mantle and emerges on the other side in a highly fragmented state. The debris then evolves into a long spiral-armlike structure that proceeds to rain down across the target over many hours. Thus, these events would allow massive impactors to stochastically deliver large quantities of HSEs, but in a manner akin to small-body accretion that optimizes emulsification into the upper mantle (16). If the largest terrestrial late accretion events were of this nature, several ongoing conundrums may be explained, including why mantle peridotites in Earth’s early rock record have surprisingly similar, although not necessarily uniform HSE abundances (29, 30) and how the iron in the projectile’s core became oxidized (9, 31).

Assuming the late accretion size distribution hitting Earth, the Moon, and Mars had $q \sim 2$, the best-case Monte Carlo results yield mean and median diameters of the largest Earth and Moon impactors of 2500 to 3000 km and 250 to 300 km, respectively. For Mars, whose formation, differentiation, and evolution are not linked in time to the Earth-Moon system, we developed an alternative Monte Carlo code. Choosing random projectiles from a size distribution and terminating each run when the net accreted mass exceeded 2.6×10^{21} kg, we found that the largest mean and median projectiles to strike Mars were 1500 to 1800 km in diameter. For impact velocities of ~ 10 km/s, this range matches the computed projectile size needed to create the proposed 10,600-by-8500-km Borealis basin (32).

Our preferred late accretion populations (those with $q \sim 2$) appear to be consistent with results from numerical simulations in which submeter objects in the protoplanetary disk are concentrated by turbulence into gravitationally bound clumps that collapse to form $D \sim 100$ -km planetesimals (33–35). Because the new planetesimals are born big, they avoid the many survival problems faced by $D = 0.001$ -km to multikilometer planetesimals, namely gas drag driving them into the Sun or disruption via impacts from numerous smaller objects embedded in the gas disk.

We produced an accretion size distribution closely resembling runs from our previous numerical experiments (33, 36), where 1.6 Earth masses of material were placed into $D = 2$ -m objects coupled to a gas disk near 1 astronomical unit (AU) and left to evolve for ~ 2 My (Fig. 3). Here, once sufficient numbers of $D \sim 100$ -km planetesimals were created, a subset of the population underwent runaway growth and developed into a shallow $q \sim 2$ size distribution that stretches from $D > 200$ - to 300-km planetesimals all the way to planetary embryo sizes. Next, we assumed the planetesimals were dynamically excited enough by gravitational interactions with planetary embryos to induce collisional evolution (Fig. 3). The objects were given new collision probabilities and impact velocities based on

numerical simulations that tracked how planetary embryos near 1 AU affect a background planetesimal population (37, 38). After ~ 10 My, we found that many $D \sim 100$ -km bodies were smashed into rubble. Larger planetesimals, however, were harder to disrupt, with the bodies finding increasing protection via their own gravity. Apparently, once a planetesimal size distribution develops a shallow branch among large objects, it is there to stay.

Observational evidence also exists to support the idea that large planetesimals in regions adjacent to the 1-AU zone had $q \sim 2$ size distributions. In the inner and central asteroid belts (with semimajor axis < 2.8 AU), the only known asteroids beyond $D > 250$ km are Ceres, Pallas, and Vesta, with $D = 975$, 544, and 530 km, respectively (Fig. 3) (39). A power-law slope determined for asteroids between $D = 250$ and 975 km yields $q \sim 1.8$, the same as those favored in Fig. 2. Notably, the outer asteroid belt follows a power-law size-frequency distribution (SFD) in the range $100 < D < 400$ km and has no shallow branch at large sizes. Although the absence of $D > 400$ -km objects may be a by-product of small number statistics and/or collisional/dynamical evolution, the other shape differences between the inner and outer main-belt size distributions imply that planetesimal-formation mechanisms change as one approaches the so-called snowline and/or that the outer asteroid belt has been contaminated by outer solar system planetesimals captured during giant planet migration (40).

Additional observational evidence comes from ancient martian impact basins, defined as $D > 300$ -km diameter craters, that formed during the earliest bombardment phases of the solar system. Their ages are unknown, though numerical simulations suggest that the oldest impact basins were produced by leftover planetesimals in the terrestrial planet region (14), whereas the youngest probably came from colliding comets and asteroids liberated from stable reservoirs during the last substantial giant planet migration event ~ 4 Ga (40). Using populations of buried and visible basins on Mars that have been previously cataloged (41), scaling relations for complex craters from (42), and impact velocities derived from (14, 18), we transformed those basins not in saturation equilibrium (namely those with $D = 700$ to 2000 km) into a projectile size distribution (Fig. 3). As before, the impactors with $D > 200$ to 300 km have $q \sim 1.8$.

Our inferred late accretion populations may have substantially modified Earth’s rotational angular momentum vector, perhaps enough to violate constraints. To test this, we tracked the effects of inelastic collisions between Earth and suites of projectiles computed from the most favorable cases shown in Fig. 2. We found that for a range of reasonable post-giant impact values (43), Earth’s rotational angular momentum changed by ~ 1 to 4%. On average, more than half of the input angular momentum was

delivered by the largest projectile in the suite, which tended to be between 2500 and 3500 km diameter. Moreover, in at least 50% of these trial cases, Earth’s obliquity was altered by 5° to 15° .

Such an obliquity change could help to explain the Moon’s orbital inclination, but only if the projectiles struck soon after the Moon-forming event. The Moon is believed to have formed in Earth’s equatorial plane, but shortly thereafter it obtained a primordial orbital inclination value of $\sim 10^\circ$ (3). Modeling work suggests that this value was produced by gravitational interactions between the Moon and the protolunar disk (44). If the protolunar disk was too short-lived to produce the full inclination, however, late terrestrial impacts could have made up the difference by altering Earth’s obliquity by up to $\sim 10^\circ$. To work, delivered HSEs could not be sequestered to Earth’s core, no easy task given the dynamic, high-temperature environment of Earth at this time, and the largest impactors would need to hit before Earth’s tides pushed the Moon beyond ~ 20 Earth radii from Earth (3). Note that the estimated interval for the latter, on the order of ~ 1 My, is short compared with numerical computations of late accretion time scales.

The Moon’s interior was once thought to be largely dry, with bulk water estimates of less than 1 part per billion (ppb) (45). Recent sample measurements, however, suggest that the water content in the lunar mantle is between 200 and several thousand ppb (or more) (45, 46). If true, it is possible that the same projectile that delivered most of the Moon’s HSEs may have also have provided it with water. Assuming that our inferred $D = 250$ - to 300-km lunar projectile could reach and mix itself into a spherical shell that is 100 to 500 km deep within the Moon and that the projectile had a minimum bulk water content of 0.05 to 0.2 weight percent (wt %) (47, 48), we estimate that 400 to 3000 ppb water could be delivered to the lunar interior by late accretion. Thus, late accretion provides an alternative explanation in case lunar mantle water cannot migrate from the post-giant impact Earth to a growing Moon through a hot and largely vaporized protolunar disk (3).

References and Notes

1. A. Borisov, H. Palme, B. Spettel, *Geochim. Cosmochim. Acta* **58**, 705 (1994).
2. J. H. Jones, M. J. Drake, *Nature* **323**, 470 (1986).
3. R. M. Canup, *Annu. Rev. Astron. Astrophys.* **42**, 441 (2004).
4. M. Touboul, T. Kleine, B. Bourdon, H. Palme, R. Wieler, *Nature* **450**, 1206 (2007).
5. P. H. Warren, in *Meteorites, Comets and Planets: Treatise on Geochemistry*, A. M. Davis, H. D. Holland, K. K. Turekian, Ed. (Elsevier, Amsterdam, 2005), vol. 1, pp. 559–599.
6. H. Becker et al., *Geochim. Cosmochim. Acta* **70**, 4528 (2006).
7. A. D. Brandon, R. J. Walker, J. W. Morgan, G. G. Goles, *Geochim. Cosmochim. Acta* **64**, 4083 (2000).
8. J. H. Jones, C. R. Neal, J. C. Ely, *Chem. Geol.* **196**, 21 (2003).
9. R. J. Walker, M. F. Horan, C. K. Shearer, J. J. Papike, *Earth Planet. Sci. Lett.* **224**, 399 (2004).
10. J. M. D. Day, D. G. Pearson, L. A. Taylor, *Science* **315**, 217 (2007).

11. J. M. D. Day, R. J. Walker, O. B. James, I. S. Puchtel, *Earth Planet. Sci. Lett.* **289**, 595 (2010).
12. R. J. Walker, *Chem. Erde* **69**, 101 (2009).
13. C.-L. Chou, *Proc. Lunar Planet. Sci. Conf.* **9**, 219 (1978).
14. W. F. Bottke, H. F. Levison, D. Nesvorný, L. Dones, *Icarus* **190**, 203 (2007).
15. By taking the ratio of Os concentration in the primitive upper mantle (~3.3 ppb) against the average concentration of all three chondrite groups weighted equally (~660 ppb) (12), we estimate that the terrestrial mass addition fraction is 0.005. Assuming the mass of Earth's mantle is 4.0×10^{24} kg, the terrestrial mass addition requirement is 2.0×10^{22} kg. A similar calculation can be made for Mars. If HSEs in the martian mantle are $0.7 \times$ the terrestrial concentration [for example, 2.4 ppb Os; consistent with the current database for shergottites (8)], and the martian mantle comprises ~80% of the mass of the planet (5.1×10^{23} kg), the martian mass addition requirement is $\sim 2.0 \times 10^{21}$ kg. This yields a ratio for terrestrial/martian additions of 10. For the Moon, assuming HSE abundances in the lunar mantle are ≤ 20 times lower than in the terrestrial mantle (9, 10), we obtain a maximum Os concentration of 0.16 ppb. Assuming the mass of the lunar mantle is 6.9×10^{22} kg and the same average chondritic concentration for the impactors, we obtain a lunar mass addition requirement of 1.7×10^{19} kg. This yields a ratio for terrestrial/lunar additions of 1200.
16. T. W. Dahl, D. J. Stevenson, *Earth Planet. Sci. Lett.* **295**, 177 (2010).
17. Late accretion of this magnitude would have had a modest effect on the W isotopic composition of Earth. The amount of impactor(s) mass envisioned to add the HSEs to the mantle would also add ~10% of the total W present in the silicate Earth (assuming that ~10% of the W presently resides in the silicate portion of Earth and that the late accretion impactor made up ~1% of the mass of the mantle). The ϵ_W value of bulk-silicate Earth, defined as a part in 10,000 deviation of $^{182}\text{W}/^{184}\text{W}$ from the terrestrial ratio, is 0. If the bulk impactor had an ϵ_W value of ~2.0 (chondritic) at the time of impact, as seems likely, mass balance requires that the ϵ_W value of the silicate Earth before impact was ~+0.2. If the silicate Earth and Moon had the same ϵ_W value and W concentrations at the time of the Moon-forming giant impact event (16), the Moon should have a slightly higher ϵ_W value after the proposed late accretion event. The current best estimate for the ϵ_W value for the Moon is $+0.09 \pm 0.10$ (2 σ) (4). Within uncertainties, this value is ambiguously identical to both the present terrestrial value and the projected pre-late accretion impact Earth.
18. W. F. Bottke *et al.*, *Icarus* **156**, 399 (2002).
19. D. Nesvorný *et al.*, *Astrophys. J.* **713**, 816 (2010).
20. N. A. Artemieva, V. V. Shuvalov, *Sol. Syst. Res.* **42**, 329 (2008).
21. We calculated an average projectile retention factor of ~30% for planetesimals composed of 50-50 ice/rock mixtures by splitting the difference between the data in (20) for stony and water-ice projectiles striking the Moon at 18 and 25 km/s, respectively. We exclude nearly isotropic comets from our analysis because they are extremely unlikely to hit Earth and the Moon in the interval between the Moon-forming impact and the solidification of the lunar crust.
22. I. S. Puchtel, R. J. Walker, O. B. James, D. A. Kring, *Geochim. Cosmochim. Acta* **72**, 3022 (2008).
23. A. N. Halliday, in *Meteorites, Comets and Planets: Treatise on Geochemistry*, A. M. Davis, H. D. Holland, K. K. Turekian, Ed. (Elsevier, Amsterdam, 2005), vol. 1, pp. 509–557.
24. Projectiles were randomly selected from a size distribution $dN \propto D^{-q}dD$. The range of D was set to 200 to 4000 km; this was designed to provide some flexibility in case the largest projectiles deliver only ~80 to 90% of their HSEs to the mantle. A successful run had an Earth/Moon mass ratio >700 with the accreted mass on Earth set to $>2.0 \times 10^{22}$ kg. The Earth/Moon impact number flux ratio was set to 20 (14, 18, 19). The sizes of the largest impactors, as well as the number of objects hitting each world within a given run were treated as variables.
25. D. C. Rubie, H. J. Melosh, J. E. Reid, C. Liebske, K. Righter, *Earth Planet. Sci. Lett.* **205**, 239 (2003).
26. D. C. Rubie, C. K. Gessmann, D. J. Frost, *Nature* **429**, 58 (2004).
27. L. E. Borg, D. S. Draper, *Meteorit. Planet. Sci.* **38**, 1713 (2003).
28. E. Asphaug, *Chem. Erde* **70**, 199 (2010).
29. V. C. Bennett, A. P. Nutman, T. M. Esat, *Geochim. Cosmochim. Acta* **66**, 2615 (2002).
30. W. D. Maier *et al.*, *Nature* **460**, 620 (2009).
31. If the proposed late accretion impactor(s) added to Earth was differentiated and contained substantial metallic iron, our model requires that the metal eventually oxidized and/or remained forever isolated from the core. This is because metal removal to the core would also lead to removal of the majority of the impactor's HSE contribution. The issue of relict metal, resulting from such an event, can be resolved via oxidation. Because the amount of metal added to the mantle by our late accretion model would comprise only ~0.3% of the total mass of the mantle (assuming that 30% of the mass of the impactor was metal and that the mass of the impactor was 1% of the mass of the mantle), it could have been oxidized by reaction with ferric iron in the early mantle. This would imply that the $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratio (where ΣFe is total Fe) of the mantle before the impact was ~0.15, rather than the present estimate of ~0.1, which is well within the range of ferrous/ferric ratios possible for the early Earth, based on chondritic comparisons.
32. M. M. Marinova, O. Aharonson, E. Asphaug, *Nature* **453**, 1216 (2008).
33. A. Morbidelli, W. F. Bottke, D. Nesvorný, H. F. Levison, *Icarus* **204**, 558 (2009).
34. A. Johansen *et al.*, *Nature* **448**, 1022 (2007).
35. J. N. Cuzzi, R. C. Hogan, W. F. Bottke, *Icarus* **208**, 518 (2010).
36. See figure 6, A and B, of (33). A detailed discussion of the code and approximations used in this simulation can be found in (33).
37. W. F. Bottke, D. Nesvorný, R. E. Grimm, A. Morbidelli, D. P. O'Brien, *Nature* **439**, 821 (2006).
38. The intrinsic collision probabilities (P_i) and impact velocities (V) of stony planetesimals striking one another near 1 AU were computed from the numerical runs described in (37). We estimate that at ~1 AU, $P_i = 60 \times 10^{-18} \text{ km}^{-2} \text{ yr}^{-1}$ and $V = 11 \text{ km/s}$.
39. P. Farinella, D. R. Davis, *Icarus* **97**, 111 (1992).
40. H. F. Levison *et al.*, *Nature* **460**, 364 (2009).
41. H. V. Frey, *J. Geophys. Res. Planets* **111**, E08591 (2006).
42. K. A. Holsapple, *Annu. Rev. Earth Planet. Sci.* **21**, 333 (1993).
43. The primordial Earth was assumed to have a spin period between 6 and 8 hours. The late accretion projectiles were assumed to hit at random locations with isotropic impact angles and impact velocities between 12 and 18 km/s.
44. W. R. Ward, R. M. Canup, *Nature* **403**, 741 (2000).
45. F. M. McCubbin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 11223 (2010).
46. Z. D. Sharp, C. K. Shearer, K. D. McKeegan, J. D. Barnes, Y. Q. Wang, *Science* **329**, 1050 (2010); 10.1126/science.1192606.
47. F. Robert, *Space Sci. Rev.* **106**, 87 (2003).
48. We chose ordinary and enstatite chondrites from (47) because they probably resemble the nature of planetesimals in the terrestrial planet region (6, 23). It is unclear, however, whether the measured water is consistent with the overall reduced nature of the rocks. The water may be from terrestrial weathering, though there are arguments against this (47). Regardless, the cited water quantities (0.05 to 0.2 wt %) are much lower than those in carbonaceous chondrite meteorites (1 to 10 wt %) (47). It is entirely possible that a $D = 250$ - to 300-km planetesimal striking the Moon during late accretion would have such bulk quantities of water.
49. We thank A. Barr, R. Canup, R. Citron, L. Dones, H. Levison, J. Jones, H. Nekvasil, A. Morbidelli, D. Minton, S. Mojszsis, G. J. Taylor, and D. Vokrouhlicky for many useful discussions and two anonymous reviewers for constructive reviews. W.F.B.'s and D.N.'s participation was supported by NASA's Lunar Science Institute through a grant to the Center for Lunar Origin and Evolution at the Southwest Research Institute in Boulder, Colorado. R.J.W.'s and J.M.D.'s work was supported by NASA's Lunar Science Institute through contract NNA09DB33A to the Center for Lunar Science and Exploration at the Lunar and Planetary Institute in Houston, Texas, and the NASA Astrobiology Program through grant NNG04G49A to the Goddard Center for Astrobiology in Greenbelt, Maryland.

24 August 2010; accepted 10 November 2010
10.1126/science.1196874

Thought for Food: Imagined Consumption Reduces Actual Consumption

Carey K. Morewedge,^{1*} Young Eun Huh,² Joachim Vosgerau²

The consumption of a food typically leads to a decrease in its subsequent intake through habituation—a decrease in one's responsiveness to the food and motivation to obtain it. We demonstrated that habituation to a food item can occur even when its consumption is merely imagined. Five experiments showed that people who repeatedly imagined eating a food (such as cheese) many times subsequently consumed less of the imagined food than did people who repeatedly imagined eating that food fewer times, imagined eating a different food (such as candy), or did not imagine eating a food. They did so because they desired to eat it less, not because they considered it less palatable. These results suggest that mental representation alone can engender habituation to a stimulus.

People believe that thinking about a desirable food or drug sensitizes one to it, increasing their hedonic response to the stimulus (1). Indeed, picturing oneself eating a delicious steak elicits an increase in salivation

and the desire to eat it (2), and imagining the sight or smell of a burning cigarette increases smokers' craving (3). The increased desirability of imagined stimuli seems to similarly affect behavior: Children have greater difficulty resisting

11. J. M. D. Day, R. J. Walker, O. B. James, I. S. Puchtel, *Earth Planet. Sci. Lett.* **289**, 595 (2010).
12. R. J. Walker, *Chem. Erde* **69**, 101 (2009).
13. C.-L. Chou, *Proc. Lunar Planet. Sci. Conf.* **9**, 219 (1978).
14. W. F. Bottke, H. F. Levison, D. Nesvorný, L. Dones, *Icarus* **190**, 203 (2007).
15. By taking the ratio of Os concentration in the primitive upper mantle (~3.3 ppb) against the average concentration of all three chondrite groups weighted equally (~660 ppb) (12), we estimate that the terrestrial mass addition fraction is 0.005. Assuming the mass of Earth's mantle is 4.0×10^{24} kg, the terrestrial mass addition requirement is 2.0×10^{22} kg. A similar calculation can be made for Mars. If HSEs in the martian mantle are $0.7 \times$ the terrestrial concentration [for example, 2.4 ppb Os; consistent with the current database for shergottites (8)], and the martian mantle comprises ~80% of the mass of the planet (5.1×10^{23} kg), the martian mass addition requirement is $\sim 2.0 \times 10^{21}$ kg. This yields a ratio for terrestrial/martian additions of 10. For the Moon, assuming HSE abundances in the lunar mantle are ≤ 20 times lower than in the terrestrial mantle (9, 10), we obtain a maximum Os concentration of 0.16 ppb. Assuming the mass of the lunar mantle is 6.9×10^{22} kg and the same average chondritic concentration for the impactors, we obtain a lunar mass addition requirement of 1.7×10^{19} kg. This yields a ratio for terrestrial/lunar additions of 1200.
16. T. W. Dahl, D. J. Stevenson, *Earth Planet. Sci. Lett.* **295**, 177 (2010).
17. Late accretion of this magnitude would have had a modest effect on the W isotopic composition of Earth. The amount of impactor(s) mass envisioned to add the HSEs to the mantle would also add ~10% of the total W present in the silicate Earth (assuming that ~10% of the W presently resides in the silicate portion of Earth and that the late accretion impactor made up ~1% of the mass of the mantle). The ϵ_W value of bulk-silicate Earth, defined as a part in 10,000 deviation of $^{182}\text{W}/^{184}\text{W}$ from the terrestrial ratio, is 0. If the bulk impactor had an ϵ_W value of ~2.0 (chondritic) at the time of impact, as seems likely, mass balance requires that the ϵ_W value of the silicate Earth before impact was ~+0.2. If the silicate Earth and Moon had the same ϵ_W value and W concentrations at the time of the Moon-forming giant impact event (16), the Moon should have a slightly higher ϵ_W value after the proposed late accretion event. The current best estimate for the ϵ_W value for the Moon is $+0.09 \pm 0.10$ (2 σ) (4). Within uncertainties, this value is ambiguously identical to both the present terrestrial value and the projected pre-late accretion impact Earth.
18. W. F. Bottke *et al.*, *Icarus* **156**, 399 (2002).
19. D. Nesvorný *et al.*, *Astrophys. J.* **713**, 816 (2010).
20. N. A. Artemieva, V. V. Shuvalov, *Sol. Syst. Res.* **42**, 329 (2008).
21. We calculated an average projectile retention factor of ~30% for planetesimals composed of 50-50 ice/rock mixtures by splitting the difference between the data in (20) for stony and water-ice projectiles striking the Moon at 18 and 25 km/s, respectively. We exclude nearly isotropic comets from our analysis because they are extremely unlikely to hit Earth and the Moon in the interval between the Moon-forming impact and the solidification of the lunar crust.
22. I. S. Puchtel, R. J. Walker, O. B. James, D. A. Kring, *Geochim. Cosmochim. Acta* **72**, 3022 (2008).
23. A. N. Halliday, in *Meteorites, Comets and Planets: Treatise on Geochemistry*, A. M. Davis, H. D. Holland, K. K. Turekian, Ed. (Elsevier, Amsterdam, 2005), vol. 1, pp. 509–557.
24. Projectiles were randomly selected from a size distribution $dN \propto D^{-q}dD$. The range of D was set to 200 to 4000 km; this was designed to provide some flexibility in case the largest projectiles deliver only ~80 to 90% of their HSEs to the mantle. A successful run had an Earth/Moon mass ratio >700 with the accreted mass on Earth set to $>2.0 \times 10^{22}$ kg. The Earth/Moon impact number flux ratio was set to 20 (14, 18, 19). The sizes of the largest impactors, as well as the number of objects hitting each world within a given run were treated as variables.
25. D. C. Rubie, H. J. Melosh, J. E. Reid, C. Liebske, K. Righter, *Earth Planet. Sci. Lett.* **205**, 239 (2003).
26. D. C. Rubie, C. K. Gessmann, D. J. Frost, *Nature* **429**, 58 (2004).
27. L. E. Borg, D. S. Draper, *Meteorit. Planet. Sci.* **38**, 1713 (2003).
28. E. Asphaug, *Chem. Erde* **70**, 199 (2010).
29. V. C. Bennett, A. P. Nutman, T. M. Esat, *Geochim. Cosmochim. Acta* **66**, 2615 (2002).
30. W. D. Maier *et al.*, *Nature* **460**, 620 (2009).
31. If the proposed late accretion impactor(s) added to Earth was differentiated and contained substantial metallic iron, our model requires that the metal eventually oxidized and/or remained forever isolated from the core. This is because metal removal to the core would also lead to removal of the majority of the impactor's HSE contribution. The issue of relict metal, resulting from such an event, can be resolved via oxidation. Because the amount of metal added to the mantle by our late accretion model would comprise only ~0.3% of the total mass of the mantle (assuming that 30% of the mass of the impactor was metal and that the mass of the impactor was 1% of the mass of the mantle), it could have been oxidized by reaction with ferric iron in the early mantle. This would imply that the $\text{Fe}^{3+}/\Sigma\text{Fe}$ ratio (where ΣFe is total Fe) of the mantle before the impact was ~0.15, rather than the present estimate of ~0.1, which is well within the range of ferrous/ferric ratios possible for the early Earth, based on chondritic comparisons.
32. M. M. Marinova, O. Aharonson, E. Asphaug, *Nature* **453**, 1216 (2008).
33. A. Morbidelli, W. F. Bottke, D. Nesvorný, H. F. Levison, *Icarus* **204**, 558 (2009).
34. A. Johansen *et al.*, *Nature* **448**, 1022 (2007).
35. J. N. Cuzzi, R. C. Hogan, W. F. Bottke, *Icarus* **208**, 518 (2010).
36. See figure 6, A and B, of (33). A detailed discussion of the code and approximations used in this simulation can be found in (33).
37. W. F. Bottke, D. Nesvorný, R. E. Grimm, A. Morbidelli, D. P. O'Brien, *Nature* **439**, 821 (2006).
38. The intrinsic collision probabilities (P_i) and impact velocities (V) of stony planetesimals striking one another near 1 AU were computed from the numerical runs described in (37). We estimate that at ~1 AU, $P_i = 60 \times 10^{-18} \text{ km}^{-2} \text{ yr}^{-1}$ and $V = 11 \text{ km/s}$.
39. P. Farinella, D. R. Davis, *Icarus* **97**, 111 (1992).
40. H. F. Levison *et al.*, *Nature* **460**, 364 (2009).
41. H. V. Frey, *J. Geophys. Res. Planets* **111**, E08591 (2006).
42. K. A. Holsapple, *Annu. Rev. Earth Planet. Sci.* **21**, 333 (1993).
43. The primordial Earth was assumed to have a spin period between 6 and 8 hours. The late accretion projectiles were assumed to hit at random locations with isotropic impact angles and impact velocities between 12 and 18 km/s.
44. W. R. Ward, R. M. Canup, *Nature* **403**, 741 (2000).
45. F. M. McCubbin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 11223 (2010).
46. Z. D. Sharp, C. K. Shearer, K. D. McKeegan, J. D. Barnes, Y. Q. Wang, *Science* **329**, 1050 (2010); 10.1126/science.1192606.
47. F. Robert, *Space Sci. Rev.* **106**, 87 (2003).
48. We chose ordinary and enstatite chondrites from (47) because they probably resemble the nature of planetesimals in the terrestrial planet region (6, 23). It is unclear, however, whether the measured water is consistent with the overall reduced nature of the rocks. The water may be from terrestrial weathering, though there are arguments against this (47). Regardless, the cited water quantities (0.05 to 0.2 wt %) are much lower than those in carbonaceous chondrite meteorites (1 to 10 wt %) (47). It is entirely possible that a $D = 250$ - to 300-km planetesimal striking the Moon during late accretion would have such bulk quantities of water.
49. We thank A. Barr, R. Canup, R. Citron, L. Dones, H. Levison, J. Jones, H. Nekvasil, A. Morbidelli, D. Minton, S. Mojszisz, G. J. Taylor, and D. Vokrouhlicky for many useful discussions and two anonymous reviewers for constructive reviews. W.F.B.'s and D.N.'s participation was supported by NASA's Lunar Science Institute through a grant to the Center for Lunar Origin and Evolution at the Southwest Research Institute in Boulder, Colorado. R.J.W.'s and J.M.D.'s work was supported by NASA's Lunar Science Institute through contract NNA09DB33A to the Center for Lunar Science and Exploration at the Lunar and Planetary Institute in Houston, Texas, and the NASA Astrobiology Program through grant NNG04G49A to the Goddard Center for Astrobiology in Greenbelt, Maryland.

24 August 2010; accepted 10 November 2010
10.1126/science.1196874

Thought for Food: Imagined Consumption Reduces Actual Consumption

Carey K. Morewedge,^{1*} Young Eun Huh,² Joachim Vosgerau²

The consumption of a food typically leads to a decrease in its subsequent intake through habituation—a decrease in one's responsiveness to the food and motivation to obtain it. We demonstrated that habituation to a food item can occur even when its consumption is merely imagined. Five experiments showed that people who repeatedly imagined eating a food (such as cheese) many times subsequently consumed less of the imagined food than did people who repeatedly imagined eating that food fewer times, imagined eating a different food (such as candy), or did not imagine eating a food. They did so because they desired to eat it less, not because they considered it less palatable. These results suggest that mental representation alone can engender habituation to a stimulus.

People believe that thinking about a desirable food or drug sensitizes one to it, increasing their hedonic response to the stimulus (1). Indeed, picturing oneself eating a delicious steak elicits an increase in salivation

and the desire to eat it (2), and imagining the sight or smell of a burning cigarette increases smokers' craving (3). The increased desirability of imagined stimuli seems to similarly affect behavior: Children have greater difficulty resisting

the impulse to eat one marshmallow immediately in order to eat two a few minutes later if they can see the marshmallow while they wait (4). Although much evidence appears to support this common intuition (4–7), its accuracy is quite puzzling, because it seems to contradict decades of research examining the overlap between direct perception and mental imagery.

Perception and mental imagery differ in their source (the senses and memory, respectively), but there is great overlap within modalities. Both engage similar neural machinery and similarly affect emotions, response tendencies, and skilled motor behavior (8–11). The thought of a spider crawling across one's leg can produce the same increases in perspiration and heart rate that would result from a spider's actual presence (5). Even the mere simulation of a motor skill can result in an improvement in its subsequent performance (9, 12). Because perception and mental imagery tend to elicit similar responses, one would expect that thinking about the consumption of a stimulus should habituate one to it.

Habituation denotes the decreased physiological and behavioral responses induced by extended or repeated exposure to a stimulus (13–15). A 10th bite of chocolate, for example, is desired less than the first bite. People habituate to a wide range of stimuli, from the brightness of a light to their income (16, 17). Habituation to food occurs too quickly for it to result from digestive feedback, so it is commonly thought to occur as a result of top-down cognitive processes (such as beliefs, memories, or expectations) or pre-ingestive sensory factors (such as texture or smell) (13, 18). Given the overlap in perception and mental representation, thinking about the consumption of a food should lead people to habituate to it.

Why then might people exhibit sensitization when thinking about a stimulus? We suggest that the sensitizing effect of imagery found in previous research may be due to the kind of imagery it has used: having participants vividly imagine a single exposure to a stimulus or associated cues. This form of imagery is more analogous to the initial exposure to a stimulus that whets the appetite and induces sensitization than to the repeated experience of a stimulus necessary to engender habituation (13). We suggest that mentally simulating an experience that is more analogous to repeated exposure (such as repeatedly imagining the consumption of units of a food) might engender habituation to the stimulus. We report five experiments testing whether repeated mental simulation of experiencing a stimulus, alone, can engender habituation. Specifically, we examined the effects of repeatedly imagining the consumption of a food on subsequent consumption of that food.

Our first experiment ($N = 51$ participants) tested whether repeatedly imagining the consumption of a food would increase or decrease the amount of that food that people would subsequently consume. All participants imagined performing 33 repetitive actions, one at a time, to hold effort constant across conditions. Controls imagined inserting 33 quarters into a laundry machine. (This served as a control task because it involves motor actions similar to those involved in eating M&M chocolate candies.) Participants in a three-repetition condition imagined inserting 30 quarters into a laundry machine and then imagined eating 3 M&M's. Participants in a 30-repetition condition imagined inserting 3 quarters into a laundry machine and then imagined eating 30 M&M's. Then all participants ate ad libitum from a bowl containing 40 g of M&M's as preparation for a "taste test" (19). The M&M's were removed when participants indicated they were finished eating. The amount of food that each participant ate was surreptitiously measured on a digital scale after the experiment. In all experiments, condition assignment was random.

A between-subjects analysis of variance (ANOVA) showed that the amount of M&M's eaten by participants was influenced by the imagination induction, $F(2, 46) = 3.61$, $P < 0.05$ (Table 1). Planned comparisons revealed that participants in the 30-repetition condition ate significantly fewer M&M's than did participants in the three-repetition condition and participants in the control condition, $F(1, 46) = 5.81$, $P < 0.05$ and $F(1, 46) = 4.50$, $P < 0.05$, respectively (Table 1). The amount eaten by participants in the three-repetition and control conditions did not differ significantly, $F < 1$, so the imagination induction did not sensitize participants to the food. Rather, repeatedly imagining the consumption of a food habituated participants to the food.

To ensure that the results of the previous experiment were due to imagined consumption rather than to the control task, in experiment 2

($N = 51$ participants), we orthogonally manipulated the experience that participants imagined (eating M&M's or the control task) and the number of times that experience was imagined (3 or 30 repetitions). A 2(repetitions: 3, 30) $\times$ 2(behavior imagined: eating, control) between-subjects ANOVA yielded a significant interaction, $F(1, 47) = 4.65$, $P < 0.05$. Planned comparisons revealed that participants who imagined eating 30 M&M's subsequently consumed fewer M&M's than did participants who imagined eating 3 M&M's, $F(1, 47) = 4.24$, $P < 0.05$. Repetitions of the control task performed did not affect subsequent consumption of M&M's, $F(1, 47) = 1.05$, $P > 0.30$. No main effects were found, F 's < 1 (Table 1).

Experiments 3 to 5 tested whether habituation or an alternative process causes repetitive imaginary consumption of a food to reduce subsequent consumption of that food. Experiment 3 ($N = 68$ participants) tested whether one must repeatedly simulate consumption of the food or whether repeated exposure to the stimulus [priming (20)] is sufficient to reduce subsequent food intake. Participants imagined eating 3 or 30 M&M's (simulating consumption) or imagined placing 3 or 30 M&M's into a bowl (priming) before consuming the candy ad libitum, as in the previous experiments.

A 2(repetitions: 3, 30) $\times$ 2(behavior imagined: eating M&M's, moving M&M's) between-subjects ANOVA on the amount of food consumed yielded a significant interaction, $F(1, 64) = 11.17$, $P < 0.05$. Planned comparisons revealed that participants who imagined eating 30 M&M's consumed significantly fewer M&M's than did participants who imagined eating 3 M&M's, $F(1, 64) = 6.10$, $P < 0.05$; whereas participants who imagined placing 30 M&M's into a bowl consumed significantly more M&M's than did participants who imagined placing 3 M&M's into a bowl, $F(1, 64) = 5.11$, $P < 0.05$. No main effects were found, F 's < 1 (Table 1). The results suggest that repetitive priming is not the process by

Table 1. Mean grams of the food consumed ad libitum after the imagery induction. Values are ± 1 SD from the mean. Means within rows that do not share the same symbol (* or †) differ significantly ($P < 0.05$). Each M&M weighed approximately 0.8 g; each cheese cube weighed approximately 4.5 g.

Imagery induction	Repetitions		
	0	3	30
Experiment 1 (food consumed: M&M's)			
Eating M&M's	4.08* $\pm$ 0.33	4.18* $\pm$ 3.26	2.21† $\pm$ 0.48
Experiment 2 (food consumed: M&M's)			
Manipulating quarters		4.31* $\pm$ 0.78	5.55* $\pm$ 3.86
Eating M&M's		5.57* $\pm$ 0.90	3.23† $\pm$ 2.20
Experiment 3 (food consumed: M&M's)			
Moving M&M's		3.87* $\pm$ 0.60	7.00† $\pm$ 0.54
Eating M&M's		7.59* $\pm$ 4.45	4.28† $\pm$ 3.05
Experiment 4 (food consumed: cheese)			
Eating M&M's		9.47* $\pm$ 0.98	11.15* $\pm$ 0.82
Eating cheese cubes		11.25* $\pm$ 0.27	6.36† $\pm$ 0.91
After test (predicted consumption: cheese)			
Eating M&M's		12.53* $\pm$ 2.61	13.60* $\pm$ 2.17
Eating cheese cubes		13.41* $\pm$ 1.81	19.21† $\pm$ 4.01

¹Department of Social and Decision Sciences, Porter Hall 208, Carnegie Mellon University, Pittsburgh, PA, USA. ²Tepper School of Business, Carnegie Mellon University, Pittsburgh, PA, USA.

*To whom correspondence should be addressed. E-mail: morewedged@cmu.edu

which the repetitive imaginary consumption of a food reduces subsequent food intake. Rather, repetitive priming appeared to sensitize participants to the food (21).

Habituation is stimulus-specific. Habituation to a food leads to diminished consumption of that food without much affecting the consumption of other foods (15, 18). To further test whether the effect of imaginary consumption engendered habituation or merely primed a feeling of “fullness,” participants in experiment 4 ($N = 41$ participants) imagined eating the food they subsequently consumed (cheddar cheese) or imagined eating a different food (M&M’s) before consuming cheddar cheese.

Participants imagined eating 3 or 30 cheddar cheese cubes or M&M’s and then ate ad libitum from a bowl containing 40 g of cheddar cheese cubes. A 2(repetitions: 3, 30) $\times$ 2(food imagined: cheddar cheese, M&M’s) between-subjects ANOVA on the amount of cheese that participants consumed yielded a significant interaction, $F(1, 37) = 4.99$, $P < 0.05$. Planned comparisons revealed that participants who imagined eating 30 cheese cubes consumed less cheese than did participants who imagined eating 3 cheese cubes, $F(1, 37) = 5.14$, $P < 0.05$. In contrast, participants who imagined eating 3 M&M’s or 30 M&M’s did not differ significantly in the amount of cheese they consumed, $F < 1$. No main effects were found, F ’s (1, 37) < 1.19 , P ’s > 0.28 (Table 1). The stimulus-specific effect of imagined consumption provides further evidence that habituation is the process by which repetitive imaginary consumption of a food leads to a reduction in its subsequent intake.

To ensure that the results of experiments 1 to 4 were not due to experimental demand, we described the design of experiment 4 to a new sample of participants ($N = 80$), who predicted average cheese consumption in each of its four conditions. Predictors correctly anticipated that

the imagined consumption of M&M’s did not influence the actual subsequent consumption of cheese, $t(79) = 0.66$, $P = 0.51$, but incorrectly predicted that participants who imagined eating 30 cubes of cheese would consume more cheese than would participants who imagined eating 3 cubes of cheese, $t(79) = 3.09$, $P < 0.01$; within-subjects ANOVA yielded a significant interaction, $F(1, 79) = 9.23$, $P < 0.01$ (Table 1).

Two psychological processes with distinct neural substrates appear to regulate food selection and intake. One process entails the hedonic responses to the food (liking or palatability) and may diminish intake through sensory-specific satiety (18). The other entails the motivation and appetitive drive to obtain it (wanting) and diminishes intake through habituation (15, 22, 23). In experiment 5 ($N = 81$ participants), we used a standard procedure to test changes in liking and wanting (23–25) to identify the process by which imagined consumption reduces food intake. Participants rated their liking for the imagined food before and after an imagination induction and played a reinforcement game, a measure of habituation (13, 15, 23–25). (Note that this method tested whether repeatedly imagining the consumption of a food leads one to feel disgusted by the food, which could also diminish consumption.)

First, all participants rated how much they liked cheddar cheese on a seven-point scale with endpoints: dislike extremely (1) and like extremely (7). Then, they imagined 33 repetitive tasks. Participants in a three-repetition condition imagined performing 30 repetitions of the control task (as in experiment 1) and then imagined eating 3 cheddar cheese cubes. Participants in a 30-repetition condition imagined performing three repetitions of the control task and then imagined eating 30 cheddar cheese cubes.

Then, all participants played the reinforcement game: Participants were shown a picture

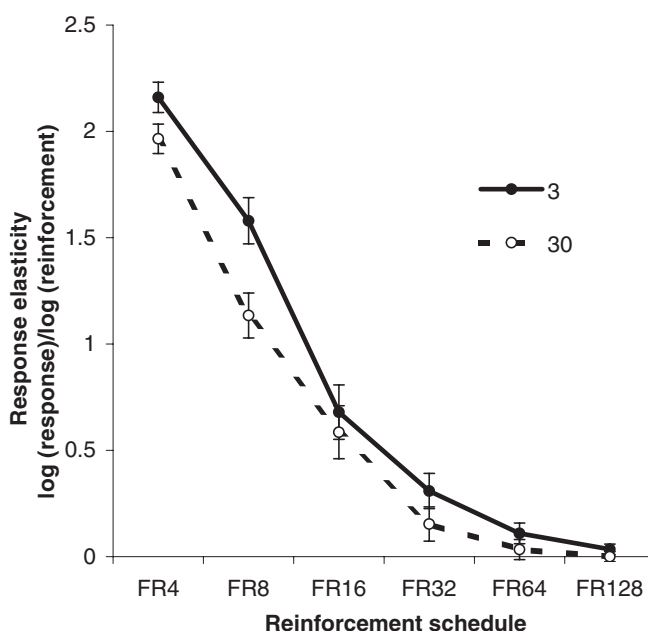
of a cheddar cheese cube and a STOP sign; they could collect points by clicking on the cheddar cheese cube. The game began with a fixed-ratio reinforcement schedule of 4 (FR4), in which every fourth click earned a point. Every time participants earned five points, the reinforcement ratio was doubled, progressing through FR8, FR16, FR32, FR64, and FR128. Participants could end the game at any time by clicking the stop sign. For each three points earned in the game, participants received one cheddar cheese cube at the end of the experiment. At the end of the game, participants re-rated how much they liked cheddar cheese on a scale identical to the scale used in the beginning of the experiment.

We calculated the difference between liking ratings before and after the imagination task to create an index of change in liking, and log-transformed responses in the reinforcement game to create an index of wanting. A 2(repetitions: 3, 30) $\times$ 2(determinant: wanting, liking) mixed ANOVA with repetitions imagined as a between-subject factor and determinant as a within-subjects factor yielded a significant main effect for determinant $F(1, 66) = 637.10$, $P < 0.01$, and a marginally significant main effect for repetition, $F(1, 66) = 3.00$, $P < 0.1$, which were qualified by a significant interaction, $F(1, 66) = 4.82$, $P < 0.05$. Planned comparisons revealed that participants who imagined eating 30 cheese cubes clicked fewer times for cheese cubes in the reinforcement game ($M_{\log \text{ response}} = 3.68 \pm 1.40$) than did participants who imagined eating 3 cheese cubes ($M_{\log \text{ response}} = 4.35 \pm 1.02$), $F(1, 66) = 5.01$, $P < 0.05$. No difference in the change of liking was observed between the two conditions ($M_{\Delta \text{ three-repetitions}} = 0.00 \pm 0.87$; $M_{\Delta \text{ thirty-repetitions}} = 0.03 \pm 0.51$), $F < 1$.

Response elasticities [responses per unit price (26)] were then computed as $[\log(\text{number of responses emitted during a specific reinforcement schedule})/\log(\text{number of reinforced responses during that reinforcement schedule})]$ and are displayed in Fig. 1. A 2(Repetitions: 3, 30) $\times$ 6(Reinforcement schedule: FR4–FR128) mixed ANOVA with repetitions as a between-subject factor and reinforcement schedules as a within-subject factor revealed that response elasticities declined linearly as reinforcement schedules progressed, $F(5, 62) = 315.08$, $P < 0.001$. As revealed in our previous analysis, participants who imagined eating 30 cheese cubes were less motivated to earn points toward cheese cubes than were participants who imagined eating 3 cheese cubes, $F(1, 66) = 4.69$, $P < 0.05$. There was a marginally significant repetitions $\times$ reinforcement schedule interaction, $F(5, 62) = 2.31$, $P < 0.1$. These results suggest that imagined consumption of a food decreased its subsequent consumption through habituation because it diminished the degree to which people wanted the food; imagined consumption did not appear to affect how much they liked it.

Together, the results show that repeatedly imagining the consumption of a food leads people to habituate to it. Participants who imagined con-

Fig. 1. Motivation to earn points for cheese (expressed as response elasticity) as a function of the number of cubes of cheese (3 versus 30) that participants had imagined eating and fixed-ratio reinforcement (for example, in FR4, every fourth response was reinforced). Error bars represent ± 1 SEM.



suming more of a food were subsequently less motivated to obtain it than were participants who imagined consuming less of the food. The influence of the imagery induction on food consumption was stimulus-specific: It reduced the consumption of the food that participants imagined eating but did not affect the consumption of other foods. Finally, repetitively imagining the consumption of the food reduced wanting (the appetitive or motivational drive) for the food. Participants who imagined consuming more of the food subsequently expended less effort to obtain it.

These findings have important implications for three fields of research. First, the results suggest that mental imagery alone can engender habituation to a stimulus. In addition to its theoretical importance, this finding may allow for the development of more effective interventions to reduce cravings for unhealthy food and drugs and diminish phobic reactions to fear-inducing stimuli. Second, it has long been debated whether pre-ingestive sensory stimulation is required for habituation to occur (13, 27–29). Previous experiments in this vein have suffered from the confound of simultaneously enacting top-down processes and pre-ingestive sensory stimulation (participants ate or smelled the food to which they habituated). Mental imagery is considered a top-down cognitive process (10, 30). Thus, the results show that top-down processes can enact habituation in the absence of pre-ingestive sensory stimulation. Finally, the results show that

repeatedly simulating an action can trigger its behavioral consequents (2). Rather than increase the likelihood of enacting the simulated behavior (eating), simulation evoked the consequences of the behavior (habituation). The difference between actual experience and mental representations of experience may be smaller than previously assumed (10).

References and Notes

1. B. Soetens, C. Braet, P. Dejonckheere, A. Roets, *J. Health Psychol.* **11**, 655 (2006).
2. M. R. Dadds, D. H. Bovbjerg, W. H. Redd, T. R. Cutmore, *Psychol. Bull.* **122**, 89 (1997).
3. S. T. Tiffany, D. J. Drobes, *Addict. Behav.* **15**, 531 (1990).
4. W. Mischel, Y. Shoda, M. I. Rodriguez, *Science* **244**, 933 (1989).
5. P. Lang, *Behav. Ther.* **8**, 862 (1977).
6. D. J. Kavanagh, J. Andrade, J. May, *Psychol. Rev.* **112**, 446 (2005).
7. E. Kemps, M. Tiggemann, *J. Exp. Psychol. Appl.* **13**, 95 (2007).
8. J. Decety, J. Grèzes, *Brain Res.* **1079**, 4 (2006).
9. J. Driskell, C. Copper, A. Moran, *J. Appl. Psychol.* **79**, 481 (1994).
10. S. M. Kosslyn, G. Ganis, W. L. Thompson, *Nat. Rev. Neurosci.* **2**, 635 (2001).
11. L. E. Williams, J. A. Bargh, *Psychol. Sci.* **19**, 302 (2008).
12. E. L. Wohlmann, A. F. Healy, L. E. Bourne Jr., *J. Exp. Psychol. Learn. Mem. Cogn.* **33**, 254 (2007).
13. F. McSweeney, S. Swindell, *Psychol. Bull.* **125**, 437 (1999).
14. F. K. McSweeney, E. S. Murphy, *J. Exp. Anal. Behav.* **74**, 347 (2000).
15. L. H. Epstein, J. L. Temple, J. N. Roemmich, M. E. Bouton, *Psychol. Rev.* **116**, 384 (2009).
16. P. Brickman, D. Coates, R. Janoff-Bulman, *J. Pers. Soc. Psychol.* **36**, 917 (1978).
17. S. Frederick, G. Loewenstein, in *Well-Being: The Foundations of Hedonic Psychology*, D. Kahneman,

- E. Diener, N. Schwarz, Eds. (Russell Sage, New York, 1999), pp. 302–329.
18. B. J. Rolls, E. T. Rolls, E. A. Rowe, K. Sweeney, *Physiol. Behav.* **27**, 137 (1981).
19. Materials and methods are available as supporting material on Science Online.
20. D. E. Huber, T. F. Clark, T. Curran, P. Winkielman, *J. Exp. Psychol. Learn. Mem. Cogn.* **34**, 1305 (2008).
21. R. Zajonc, *Am. Psychol.* **35**, 151 (1980).
22. K. C. Berridge, *Neurosci. Biobehav. Rev.* **20**, 1 (1996).
23. L. H. Epstein et al., *Appetite* **41**, 283 (2003).
24. R. C. Havermans, T. Janssen, J. C. Giesen, A. Roefs, A. Jansen, *Appetite* **52**, 222 (2009).
25. M. Myers Ernst, L. H. Epstein, *Appetite* **38**, 224 (2002).
26. R. J. DeGrandpre, W. K. Bickel, J. R. Hughes, M. P. Layng, G. Badger, *J. Exp. Anal. Behav.* **60**, 641 (1993).
27. S. Higgs, A. C. Williamson, P. Rotshtein, G. W. Humphreys, *Psychol. Sci.* **19**, 623 (2008).
28. P. Rozin, S. Dow, M. Moscovitch, S. Rajaram, *Psychol. Sci.* **9**, 392 (1998).
29. B. J. Rolls, E. A. Rowe, E. T. Rolls, *Physiol. Behav.* **29**, 409 (1982).
30. F. Crick, C. Koch, *Nature* **375**, 121 (1995).
31. This work benefited from financial support from a grant to C.K.M. from the Berkman Faculty Development Fund at Carnegie Mellon University. We thank A. Kim, K. Khanna, M. Fei, L. Mosca, M. Christian, and Center for Behavioral Decision Research summer interns in 2008 and 2009 for help with the data collection. We thank C. Janiszewski, D. Kahneman, G. Loewenstein, B. Rolls, and L. Williams for helpful comments.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1530/DC1
SOM Text
References

27 July 2010; accepted 28 October 2010
10.1126/science.1195701

Sex Determination in the Social Amoeba *Dictyostelium discoideum*

Gareth Bloomfield,^{1,2*} Jason Skelton,² Alasdair Ivens,² Yoshimasa Tanaka,^{1,3} Robert R. Kay¹

The genetics of sex determination remain mysterious in many organisms, including some that are otherwise well studied. Here we report the discovery and analysis of the mating-type locus of the model organism *Dictyostelium discoideum*. Three forms of a single genetic locus specify this species' three mating types: two versions of the locus are entirely different in sequence, and the third resembles a composite of the other two. Single, unrelated genes are sufficient to determine two of the mating types, whereas homologs of both these genes are required in the composite type. The key genes encode polypeptides that possess no recognizable similarity to established protein families. Sex determination in the social amoebae thus appears to use regulators that are unrelated to any others currently known.

Most eukaryotes are sexual, but little is known in molecular detail about sex across most branches of the eukaryotic tree. One aspect, the genetic basis of sex determination, is well understood in several animal,

fungal, and plant lineages (1–5), but across the protozoan kingdoms we know little, and nothing in comparable detail. The social amoebae are members of the Amoebozoa and have an unusual sexual cycle that leads to the formation of dormant walled macrocysts (6) (Fig. 1, A and B). To produce a macrocyst, a pair of haploid amoebae of different sexes fuse (7) to form a diploid zygote, which then attracts surrounding haploid cells (8). These help to lay down external layers of cellulose around the developing mass of cells before being cannibalized by the zygote (9). After a period of dormancy,

the cyst germinates, releasing haploid progeny that arise most likely after meiosis and multiple mitoses (10). The population genetics of wild isolates indicate that mating and recombination are probably frequent in the wild (11).

The most-studied species of social amoeba, *Dictyostelium discoideum*, is notable for having three sexes [hereafter called mating types I, II, and III [supporting online material (SOM) text S1], as well as uncommon self-fertile homothallic strains (12–14). Each of the three sexes can pair with each of the other two but not with itself, giving three possible classes of zygote: type I/type II, type I/type III, and type II/type III. Although several genes are known to be involved during the sexual cycle (15), the determinant of mating type has proved elusive. Genetic analysis suggested that mating type is stable and is determined by a single locus with two or more alleles (10, 14, 16). We argued that it might be possible to identify this postulated locus by searching for genes that are present in any member of one mating type but absent or highly diverged in any member of another. For this purpose, we performed comparative genomic hybridizations using DNA microarrays composed of probes for around 8500 of the 10,500 predicted genes in the sequenced type I *D. discoideum* genome (17).

We analyzed 10 strains derived from independent wild isolates (table S1) using this micro-

¹Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK. ²Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK. ³Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan.

*To whom correspondence should be addressed. E-mail: garethb@mrc-lmb.cam.ac.uk

suming more of a food were subsequently less motivated to obtain it than were participants who imagined consuming less of the food. The influence of the imagery induction on food consumption was stimulus-specific: It reduced the consumption of the food that participants imagined eating but did not affect the consumption of other foods. Finally, repetitively imagining the consumption of the food reduced wanting (the appetitive or motivational drive) for the food. Participants who imagined consuming more of the food subsequently expended less effort to obtain it.

These findings have important implications for three fields of research. First, the results suggest that mental imagery alone can engender habituation to a stimulus. In addition to its theoretical importance, this finding may allow for the development of more effective interventions to reduce cravings for unhealthy food and drugs and diminish phobic reactions to fear-inducing stimuli. Second, it has long been debated whether pre-ingestive sensory stimulation is required for habituation to occur (13, 27–29). Previous experiments in this vein have suffered from the confound of simultaneously enacting top-down processes and pre-ingestive sensory stimulation (participants ate or smelled the food to which they habituated). Mental imagery is considered a top-down cognitive process (10, 30). Thus, the results show that top-down processes can enact habituation in the absence of pre-ingestive sensory stimulation. Finally, the results show that

repeatedly simulating an action can trigger its behavioral consequents (2). Rather than increase the likelihood of enacting the simulated behavior (eating), simulation evoked the consequences of the behavior (habituation). The difference between actual experience and mental representations of experience may be smaller than previously assumed (10).

References and Notes

1. B. Soetens, C. Braet, P. Dejonckheere, A. Roets, *J. Health Psychol.* **11**, 655 (2006).
2. M. R. Dadds, D. H. Bovbjerg, W. H. Redd, T. R. Cutmore, *Psychol. Bull.* **122**, 89 (1997).
3. S. T. Tiffany, D. J. Drobes, *Addict. Behav.* **15**, 531 (1990).
4. W. Mischel, Y. Shoda, M. I. Rodriguez, *Science* **244**, 933 (1989).
5. P. Lang, *Behav. Ther.* **8**, 862 (1977).
6. D. J. Kavanagh, J. Andrade, J. May, *Psychol. Rev.* **112**, 446 (2005).
7. E. Kemps, M. Tiggemann, *J. Exp. Psychol. Appl.* **13**, 95 (2007).
8. J. Decety, J. Grèzes, *Brain Res.* **1079**, 4 (2006).
9. J. Driskell, C. Copper, A. Moran, *J. Appl. Psychol.* **79**, 481 (1994).
10. S. M. Kosslyn, G. Ganis, W. L. Thompson, *Nat. Rev. Neurosci.* **2**, 635 (2001).
11. L. E. Williams, J. A. Bargh, *Psychol. Sci.* **19**, 302 (2008).
12. E. L. Wohlmann, A. F. Healy, L. E. Bourne Jr., *J. Exp. Psychol. Learn. Mem. Cogn.* **33**, 254 (2007).
13. F. McSweeney, S. Swindell, *Psychol. Bull.* **125**, 437 (1999).
14. F. K. McSweeney, E. S. Murphy, *J. Exp. Anal. Behav.* **74**, 347 (2000).
15. L. H. Epstein, J. L. Temple, J. N. Roemmich, M. E. Bouton, *Psychol. Rev.* **116**, 384 (2009).
16. P. Brickman, D. Coates, R. Janoff-Bulman, *J. Pers. Soc. Psychol.* **36**, 917 (1978).
17. S. Frederick, G. Loewenstein, in *Well-Being: The Foundations of Hedonic Psychology*, D. Kahneman,

- E. Diener, N. Schwarz, Eds. (Russell Sage, New York, 1999), pp. 302–329.
18. B. J. Rolls, E. T. Rolls, E. A. Rowe, K. Sweeney, *Physiol. Behav.* **27**, 137 (1981).
19. Materials and methods are available as supporting material on Science Online.
20. D. E. Huber, T. F. Clark, T. Curran, P. Winkielman, *J. Exp. Psychol. Learn. Mem. Cogn.* **34**, 1305 (2008).
21. R. Zajonc, *Am. Psychol.* **35**, 151 (1980).
22. K. C. Berridge, *Neurosci. Biobehav. Rev.* **20**, 1 (1996).
23. L. H. Epstein *et al.*, *Appetite* **41**, 283 (2003).
24. R. C. Havermans, T. Janssen, J. C. Giesen, A. Roefs, A. Jansen, *Appetite* **52**, 222 (2009).
25. M. Myers Ernst, L. H. Epstein, *Appetite* **38**, 224 (2002).
26. R. J. DeGrandpre, W. K. Bickel, J. R. Hughes, M. P. Layng, G. Badger, *J. Exp. Anal. Behav.* **60**, 641 (1993).
27. S. Higgs, A. C. Williamson, P. Rotshtein, G. W. Humphreys, *Psychol. Sci.* **19**, 623 (2008).
28. P. Rozin, S. Dow, M. Moscovitch, S. Rajaram, *Psychol. Sci.* **9**, 392 (1998).
29. B. J. Rolls, E. A. Rowe, E. T. Rolls, *Physiol. Behav.* **29**, 409 (1982).
30. F. Crick, C. Koch, *Nature* **375**, 121 (1995).
31. This work benefited from financial support from a grant to C.K.M. from the Berkman Faculty Development Fund at Carnegie Mellon University. We thank A. Kim, K. Khanna, M. Fei, L. Mosca, M. Christian, and Center for Behavioral Decision Research summer interns in 2008 and 2009 for help with the data collection. We thank C. Janiszewski, D. Kahneman, G. Loewenstein, B. Rolls, and L. Williams for helpful comments.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1530/DC1
SOM Text
References

27 July 2010; accepted 28 October 2010
10.1126/science.1195701

Sex Determination in the Social Amoeba *Dictyostelium discoideum*

Gareth Bloomfield,^{1,2*} Jason Skelton,² Alasdair Ivens,² Yoshimasa Tanaka,^{1,3} Robert R. Kay¹

The genetics of sex determination remain mysterious in many organisms, including some that are otherwise well studied. Here we report the discovery and analysis of the mating-type locus of the model organism *Dictyostelium discoideum*. Three forms of a single genetic locus specify this species' three mating types: two versions of the locus are entirely different in sequence, and the third resembles a composite of the other two. Single, unrelated genes are sufficient to determine two of the mating types, whereas homologs of both these genes are required in the composite type. The key genes encode polypeptides that possess no recognizable similarity to established protein families. Sex determination in the social amoebae thus appears to use regulators that are unrelated to any others currently known.

Most eukaryotes are sexual, but little is known in molecular detail about sex across most branches of the eukaryotic tree. One aspect, the genetic basis of sex determination, is well understood in several animal,

fungal, and plant lineages (1–5), but across the protozoan kingdoms we know little, and nothing in comparable detail. The social amoebae are members of the Amoebozoa and have an unusual sexual cycle that leads to the formation of dormant walled macrocysts (6) (Fig. 1, A and B). To produce a macrocyst, a pair of haploid amoebae of different sexes fuse (7) to form a diploid zygote, which then attracts surrounding haploid cells (8). These help to lay down external layers of cellulose around the developing mass of cells before being cannibalized by the zygote (9). After a period of dormancy,

the cyst germinates, releasing haploid progeny that arise most likely after meiosis and multiple mitoses (10). The population genetics of wild isolates indicate that mating and recombination are probably frequent in the wild (11).

The most-studied species of social amoeba, *Dictyostelium discoideum*, is notable for having three sexes [hereafter called mating types I, II, and III [supporting online material (SOM) text S1], as well as uncommon self-fertile homothallic strains (12–14). Each of the three sexes can pair with each of the other two but not with itself, giving three possible classes of zygote: type I/type II, type I/type III, and type II/type III. Although several genes are known to be involved during the sexual cycle (15), the determinant of mating type has proved elusive. Genetic analysis suggested that mating type is stable and is determined by a single locus with two or more alleles (10, 14, 16). We argued that it might be possible to identify this postulated locus by searching for genes that are present in any member of one mating type but absent or highly diverged in any member of another. For this purpose, we performed comparative genomic hybridizations using DNA microarrays composed of probes for around 8500 of the 10,500 predicted genes in the sequenced type I *D. discoideum* genome (17).

We analyzed 10 strains derived from independent wild isolates (table S1) using this micro-

¹Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK. ²Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK. ³Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan.

*To whom correspondence should be addressed. E-mail: garethb@mrc-lmb.cam.ac.uk

array (18) and found a single candidate gene that follows the pattern expected of a sex-determining sequence: It is present in all type I genomes but absent from all of the type II strains (Fig. 2, fig. S1, and table S2). This open reading frame (ORF) is situated on chromosome 5 (SOM text S2) and is very short, encoding a 107-amino acid polypeptide that contains no significant homology to previously studied proteins. No motifs or potential domains suggest a function, but it is relatively highly charged and so is most likely a soluble intracellular protein. We confirmed that the ORF is present in all the other type I strains used in the microarray study (100% identical in amino acid sequence in all cases; table S3), and sequenced the entire locus from another type I strain, WS205, to confirm that no other obvious coding sequences are present.

To test whether the gene is involved in the sexual cycle, we deleted it from our standard type I strain (19). The resulting mutant, in contrast to its parent, is unable to form macrocysts when paired with a type II strain (Fig. 3). When the coding sequence is reintroduced under the control of a constitutive promoter, mating competence is restored (Fig. 3D and table S4), showing that this gene, which we call *matA*, is necessary for correct type I mating behavior, and supporting the proposition that it is involved in sex determination.

The two genes flanking *matA* in the reference type I sequence are present in all strains and do not vary in amino acid sequence between mating types (fig. S2). We could therefore amplify the entire type II version of the locus by polymerase chain reaction from the NC66.2 isolate. The type II version is larger and contains three genes (Fig. 4A), one of which, *matB*, is homologous to *matA* but considerably diverged at approximately 60% identity in amino acid sequence (fig. S3 and SOM text S1). A second small gene, *matC*, encodes a 208-amino acid hydrophilic polypeptide that again has no similarity to other known proteins (fig. S4). The third gene, *matD*, is larger, spliced, and encodes a 799-amino acid preprotein that contains a predicted signal peptide and potential glycosylphosphatidylinositol-attachment site (fig. S5). No homology is shared between these three genes. All of the other type II strains tested contain the same three genes, with >98% sequence identity (table S4), and the complete type II locus from the Japanese type II isolate NYA64 was sequenced, again confirming that no additional coding sequences are present.

To prove that this locus is responsible for sex determination, we attempted to switch the mating type of our type I laboratory strain. The *matA* gene was first deleted and the resulting mutant then transformed with a construct bearing the entire type II version of the locus. The resulting strain then behaved as type II (SOM text S3), forming macrocysts when paired with type I cells but not with type II (table S5). This reversal of mating orientation demonstrates that this locus is sufficient to specify mating types I and II and that we have identified the *Dictyostelium* mating-type locus.

Next we characterized the locus in the type III strain WS2162. This version contains two genes homologous to *matC* and *matD* but divergent in sequence, and which we name *matS* and *matT*, respectively (Fig. 4A and figs. S4 and S5). No sequence related to *matA* or *matB* is present. The

same pattern is followed in another type III isolate, WS112B (table S6). Each mating type thus possesses a different version of the mating-type locus, and there is no overlap between the type I and type III versions. The type II version resembles a composite of the other two, containing

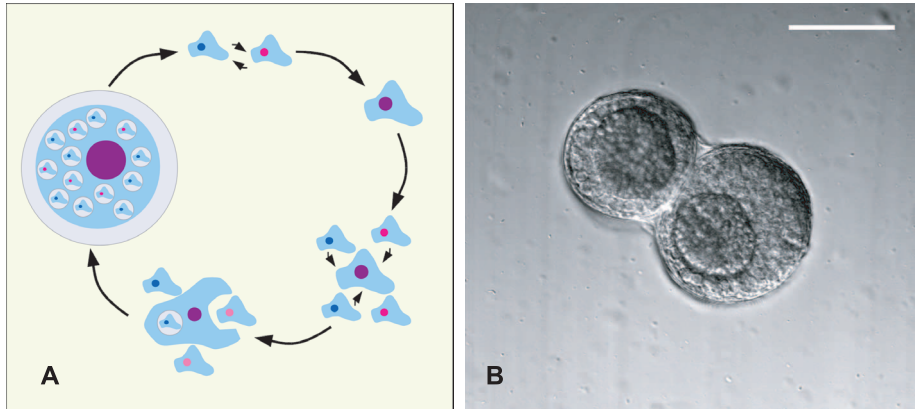
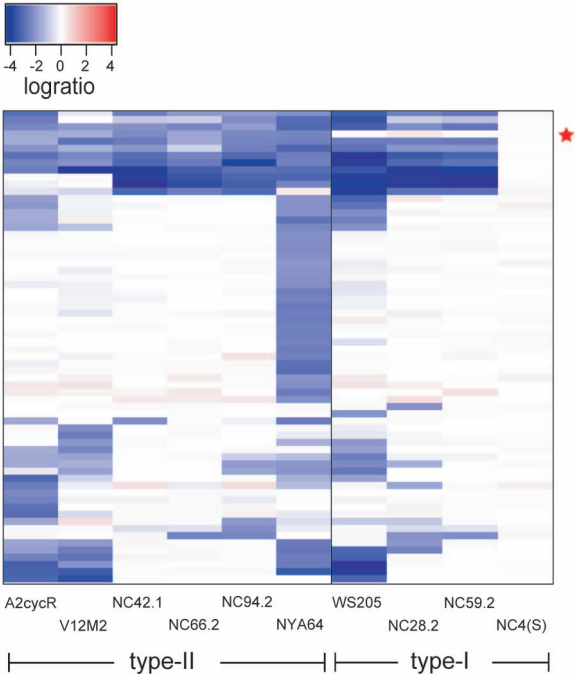


Fig. 1. The sexual cycle of *D. discoideum*. (A) Amoebae of different sexes (top) first fuse, and several hours later their two nuclei also fuse, resulting in a diploid zygote (top right). This then secretes cyclic adenosine monophosphate to attract surrounding haploid cells (bottom right), which are ingested by the zygote (bottom); eventually a dormant macrocyst is formed, retaining partly digested haploids within vacuoles (left). Ultimately the macrocyst germinates, releasing tens or hundreds of progeny, all descending from the zygote. (B) Early macrocysts (precysts) of strain AC4, formed in shaken suspension approximately 24 hours after removal of food bacteria. Here, two developing macrocysts are contained within the same outer wall. In each cyst, inner walls envelop hundreds of haploid amoebae and the zygote, which is visible here as the darker cell mass at the center of each cyst, and which slowly eats its way out to the limiting wall. Within the zygotes, the structures of ingested amoebae are still clear. Scale bar, 50 μ m.

Fig. 2. Identification of a candidate mating-type locus. Our search assumed that the mating-type locus would differ substantially in sequence between mating types, so using a microarray designed from type I sequence, we sought genes that were present in all examples of this mating type and absent from or highly diverged in all examples of type II. Out of more than 8500 genes covered on the array, only one, *matA* (alongside the red star), behaved in this way when 10 independent wild isolates were compared with our laboratory strain Ax2 in DNA-to-DNA comparisons (strain names are shown at the bottom). The heat map shows only the set of genes giving a log ratio below -2 in at least one of the wild strains as compared to Ax2 (full data are presented in table S2). Each row in the plot represents a gene, each column a strain. Blocks are colored according to $\log_2$ ratio, from blue (negative: decreased copy number or sequence divergence in the test strain) through white (zero: no difference) to red (positive: increased copy number in the test strain). Several other sequences apart from *matA* were absent or diverged in different isolates, but none of these correlates with mating type; it should be noted that NC4 is the ultimate parent of Ax2, accounting for the similarity between them.



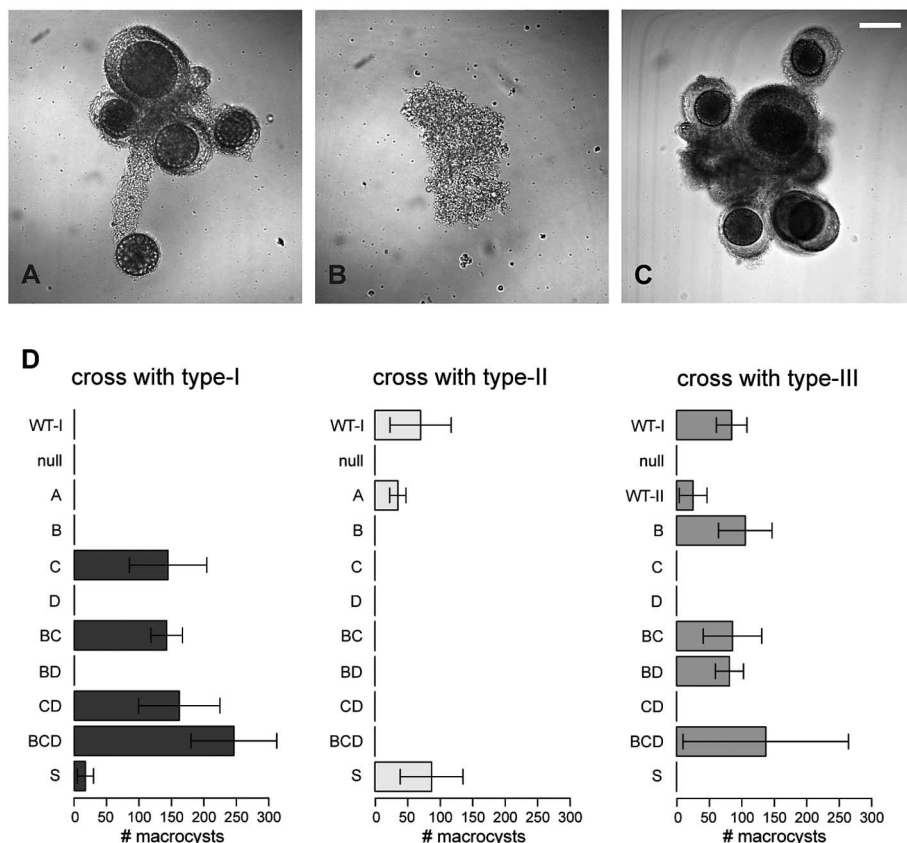


Fig. 3. Re-engineering the mating behavior of a type I strain. The type I strain Ax2, which can mate with type II strain V12M2 (A), was first modified by the deletion of *matA*. The resultant strain is unable to mate with V12M2 (B) or any other strain. The introduction of *matC* with its own regulatory sequences into this null mutant gives a strain that is able to mate with its ultimate parent Ax2 (C). Structures were imaged 7 days after mixing, by means of differential interference contrast microscopy. Scale bar, 50 μ m. (D) Macrocysts formed in various crosses were counted 8 days after mixture of strains. Eleven strains were crossed with a type I strain, Ax2 (left), and with a type II strain, V12M2 (middle): WT-I is the parental Ax2 strain and “null” is the *matA* deletion strain in this background. The next nine strains are the null plus one or more *mat* genes controlled by a constitutive promoter; each is designated by the gene’s letter. These strains, apart from the *matA*-expressing strain, and also the type II strain V12M2 (WT-II), were also crossed with the type III strain WS2162 (right). The mean number of macrocysts plus and minus the standard error from three independent crosses are plotted.

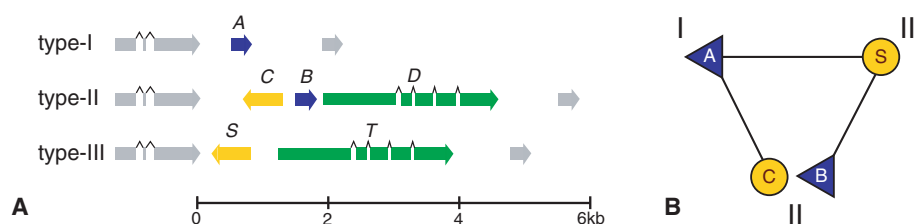


Fig. 4. The structure and logic of the *D. discoideum* *mat* locus. (A) Type I strains are characterized by a single protein-coding gene, *matA* (blue, A; dictyBase identification no. DDB_G0289165), which is homologous to *matB* (also blue, B), one of the three genes present in the type II version of the locus. The two other genes making up the type II locus, *matC* (yellow, C) and *matD* (green, D), are homologous to the two genes that occupy the type III version, *matS* and *matT* (yellow and green according to homology, S and T; gene nomenclature is treated further in the SOM text). The locus lies on chromosome 5 between the genes DDB_G0289171 and DDB_G0289163, which do not vary according to mating type and are shown here in gray. (B) Mating compatibility requires the presence of a *matA*-class gene (blue triangles) and a *matS*-class gene (yellow circles) in the two gametes. Type II cells contain a gene of each class, allowing mating with both types I and III. The nature of the interactions between genes remains unknown, as does the molecular explanation of how the *matB* and *matC* pair are incompatible.

homologs of the genes from the type I and type III versions. Two homothallic isolates resemble type III, containing genes related to *matS* and *matT* but no *matA/matB* homolog (table S6 and SOM text S4).

Because mating type I is determined by a single gene, we next asked whether any of the genes at the type II and type III versions of the locus could act as master regulators of sex determination, with others perhaps having subsidiary roles. To do this, we expressed individual genes, either alone or in combination, in the *matA* null strain, which is unable to mate with any strain. The type II locus was analyzed in most detail. Expressing *matB* alone from a constitutive promoter is sufficient to produce a strain able to mate with a type III strain, but not with types I and II (Fig. 3D and table S5). Thus, the homologous *matA* and *matB* genes appear to have similar but not identical functions: The former specifies mating competence toward type II and type III, whereas the latter is only effective toward type III, and *matB* only partially accounts for the type II phenotype.

Expressing *matC* alone in the same null background allows mating with type I cells but not type III or type II cells (Fig. 3D and table S5). We obtained the same result when we replaced the knocked-out version of the *matA* locus with a truncation of the NC66.2 *mat* locus containing only *matC* and its native regulatory sequences; again, this strain, which now contained only *matC* in place at the mating locus on chromosome 5, mated efficiently with type I cells but not at all with type III or type II cells (Fig. 3C and table S5). When the knocked-out version is replaced with the genomic region bearing both *matB* and *matC*, under the control of their own promoters, the resulting strain can form macrocysts with both types I and III (table S5). Similarly, overexpression of both *matB* and *matC* in the same strain recapitulates normal type II behavior as adjudged by this assay (Fig. 3D and table S5). These strains are not self-fertile (table S5). Therefore, two unrelated *mat* genes together specify the type II mating type.

The final gene contained in the type II version of the locus, *matD*, is not required for mating-type determination. When expressed alone in the mating-null cells from a constitutive promoter, it does not allow mating with any of the mating types, and it does not affect the qualitative behavior of *matB* or *matC* when expressed in combination with them, although there is some evidence of quantitative effects. Yields of macrocysts are higher when *matD* is present in some crosses (table S5); this is consistent with a possible role in promoting gamete fusion.

Turning to the type III version, the presence of a *matC* homolog suggested that this gene, *matS*, might specify this third mating type. As expected, expressing *matS* in the *matA* null background gives a strain that mates with type I and type II but not with type III (Fig. 3D and table S5). Furthermore, crosses between strains bearing just the master control genes give the expected results:

Null cells expressing just *matS* can mate with cells expressing just *matB* but not with cells expressing just *matC* (table S5). Finally, cells separately expressing *matB* and *matC* do not mate with each other (table S5). We have not analyzed *matT* separately, but like *matD* it does not appear to be necessary for sex determination.

These results suggest a simple underlying picture: type I and type III mating behavior can be specified by a single gene in each case: *matA* specifies type I and *matS* specifies type III. Type II is a composite in which homologs of *matA* and *matS* (*matB* and *matC*, respectively) allow it to mate with the other two mating types but, for reasons that remain unclear, not with itself (Fig. 4B).

The molecular function of these genes remains to be addressed. No clear homologs are present in species outside of the dictyostelids, but *D. purpureum* possesses two adjacent genes that are homologous to *matS* and *matT* (figs. S4 and S5). A clear *matD/matT* homolog is also evident in the more distantly related dictyostelid *Acytostelium subglobosum* (fig. S5). Adjacent to this gene is a small ORF very weakly similar to *matC/matS*, a possible *matS* ortholog in this species (fig. S6). *MatD* is distantly related to the Hap2-GCS1 family of gamete fusion proteins (20), supporting the idea that *MatD* and *MatT* are involved during fusion, although another *D. discoideum* gene, *hapA*, encodes a protein much more closely related to the canonical Hap2 group and is enriched in cells competent for mating (21).

The organization of the *Dictyostelium* mating-type locus does not closely resemble previously studied sex-determining regions (SOM text S5), although like them the *mat* locus must ultimately control a transcriptional cascade (21). Whether the

key genes directly regulate transcription or do so through downstream targets remains to be determined. One further role could be in controlling mitochondrial inheritance, which is uniparental in related Amoebozoans (22, 23).

We have identified the sex-determining locus from *D. discoideum*, a model organism and the best-studied member of the Amoebozoa. The master regulators of sex determination at this locus encode homologs of two small, apparently soluble proteins, which are unrelated to previously studied proteins. The genetic logic of the system allows one to speculate that one sex, mating type II, may have arisen after a fusion of the versions of the locus of the other two sexes. Understanding the mating-type locus may help to overcome longstanding difficulties in making use of sexual genetics in *Dictyostelium* (24).

References and Notes

1. I. Marín, B. S. Baker, *Science* **281**, 1990 (1998).
2. C. A. Smith et al., *Nature* **461**, 267 (2009).
3. S. C. Lee, M. Ni, W. Li, C. Shertz, J. Heitman, *Microbiol. Mol. Biol. Rev.* **74**, 298 (2010).
4. M. Tanurdzic, J. A. Banks, *Plant Cell* **16** (suppl.), S61 (2004).
5. A. Martin et al., *Nature* **461**, 1135 (2009).
6. J. C. Blaskovics, K. B. Raper, *Biol. Bull.* **113**, 58 (1957).
7. Y. Saga, H. Okada, K. Yanagisawa, *J. Cell Sci.* **60**, 157 (1983).
8. D. H. O'Day, *Can. J. Microbiol.* **25**, 1416 (1979).
9. M. F. Filosa, R. E. Dengler, *Dev. Biol.* **29**, 1 (1972).
10. M. A. Wallace, K. B. Raper, *J. Gen. Microbiol.* **113**, 327 (1979).
11. J. M. Flowers et al., *PLoS Genet.* **6**, e1001013 (2010).
12. L. D. Hurst, *Proc. Biol. Sci.* **263**, 415 (1996).
13. G. W. Erdos, K. B. Raper, L. K. Vogen, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1828 (1973).
14. G. E. Robson, K. L. Williams, *Curr. Genet.* **1**, 229 (1980).
15. H. Urushihara, T. Muramoto, *Eur. J. Cell Biol.* **85**, 961 (2006).
16. G. E. Robson, K. L. Williams, *Genetics* **93**, 861 (1979).

17. L. Eichinger et al., *Nature* **435**, 43 (2005).
18. G. Bloomfield, Y. Tanaka, J. Skelton, A. Ivens, R. R. Kay, *Genome Biol.* **9**, R75 (2008).
19. Materials and methods are available as supporting material on Science Online.
20. J. L. Wong, M. A. Johnson, *Trends Cell Biol.* **20**, 134 (2010).
21. T. Muramoto et al., *Mech. Dev.* **120**, 965 (2003).
22. S. Kawano, R. W. Anderson, T. Nanba, T. Kuroiwa, *J. Gen. Microbiol.* **133**, 3175 (1987).
23. M. Mirfakhrai, Y. Tanaka, K. Yanagisawa, *Genetics* **124**, 607 (1990).
24. K. B. Raper, *The Dictyostelids* (Princeton Univ. Press, Princeton, NJ, 1984), chap. 9.
25. This work was supported by Wellcome Trust grant number 06724 and core funding from the Medical Research Council. We thank the Dicty Stock Center (www.dictybase.org/StockCenter/StockCenter.html), D. Francis, H. Hagiwara, and P. Schaap for providing strains; T. Feltwell, N. Nikolaidou-Katsaridou, and K. Jagels for technical assistance; and M. Fookes, C. Carret, K. Brooks, C. Pears, N. Barry, and the members and associate members of the *Dictyostelium* group at the Laboratory of Molecular Biology for valuable advice and discussions. A.I. is now at Fios Genomics, ETT, King's Buildings, Edinburgh EH9 3JL, UK. We are grateful to the U.S. Department of Energy Joint Genome Institute (www.jgi.doe.gov/) and co-workers, and to the *Acytostelium* Genome Consortium for making the *Dictyostelium purpureum* and *Acytostelium subglobosum* genome data available before publication. Microarray data are available under accession nos. E-TABM-394 and the array design under A-SGRP-3 at www.ebi.ac.uk/arrayexpress/, and DNA sequences have been deposited in EMBL-Bank under accessions nos. FN543120 to FN543124, FN994780, FR666792, and FR666793.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1533/DC1
Materials and Methods
SOM Text S1 to S5
Figs. S1 to S6
Tables S1 to S6
References

6 September 2010; accepted 28 October 2010
10.1126/science.1197423

Nebulin and N-WASP Cooperate to Cause IGF-1–Induced Sarcomeric Actin Filament Formation

Kazunori Takano,^{1,2} Haruko Watanabe-Takano,^{1,2} Shiro Suetsugu,^{2,3,4} Souichi Kurita,^{1*} Kazuya Tsujita,⁵ Sumiko Kimura,¹ Takashi Karatsu,⁶ Tadaomi Takenawa,^{2,5} Takeshi Endo^{1,2†}

Insulin-like growth factor 1 (IGF-1) induces skeletal muscle maturation and enlargement (hypertrophy). These responses require protein synthesis and myofibril formation (myofibrillogenesis). However, the signaling mechanisms of myofibrillogenesis remain obscure. We found that IGF-1–induced phosphatidylinositol 3-kinase–Akt signaling formed a complex of nebulin and N-WASP at the Z bands of myofibrils by interfering with glycogen synthase kinase-3 β in mice. Although N-WASP is known to be an activator of the Arp2/3 complex to form branched actin filaments, the nebulin–N-WASP complex caused actin nucleation for unbranched actin filament formation from the Z bands without the Arp2/3 complex. Furthermore, N-WASP was required for IGF-1–induced muscle hypertrophy. These findings present the mechanisms of IGF-1–induced actin filament formation in myofibrillogenesis required for muscle maturation and hypertrophy and a mechanism of actin nucleation.

IGF-1–induced muscle maturation and hypertrophy necessitate not only protein synthesis but also myofibrillogenesis. IGF-1 causes pro-

tein synthesis by activating phosphatidylinositol 3-kinase (PI3K)–Akt signaling (1, 2). In contrast, the signaling mechanisms of myofibrillogenesis

are unclear. Although the proteins inducing actin filament formation in nonmuscle cells have been elucidated (3–7), those responsible for actin filament formation from the Z bands of myofibrils remain unknown. Thus, we asked whether N-WASP, which causes rapid actin polymerization by activating the Arp2/3 complex in nonmuscle cells, is involved in myofibrillar actin filament formation.

¹Department of Biology, Graduate School of Science, Chiba University, 1-33 Yayoicho, Inageku, Chiba 263-8522, Japan.

²Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), Chiyodaku, Tokyo 102-0075, Japan. ³Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo 113-0032, Japan. ⁴Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency (JST), Chiyodaku, Tokyo 102-0075, Japan. ⁵Division of Lipid Biochemistry, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan. ⁶Department of Applied Chemistry and Biotechnology, Graduate School of Engineering, Chiba University, 1-33 Yayoicho, Inageku, Chiba 263-8522, Japan.

*Present address: Division of Molecular and Cellular Biology, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan.

†To whom correspondence should be addressed. E-mail: t.endo@faculty.chiba-u.jp

Null cells expressing just *matS* can mate with cells expressing just *matB* but not with cells expressing just *matC* (table S5). Finally, cells separately expressing *matB* and *matC* do not mate with each other (table S5). We have not analyzed *matT* separately, but like *matD* it does not appear to be necessary for sex determination.

These results suggest a simple underlying picture: type I and type III mating behavior can be specified by a single gene in each case: *matA* specifies type I and *matS* specifies type III. Type II is a composite in which homologs of *matA* and *matS* (*matB* and *matC*, respectively) allow it to mate with the other two mating types but, for reasons that remain unclear, not with itself (Fig. 4B).

The molecular function of these genes remains to be addressed. No clear homologs are present in species outside of the dictyostelids, but *D. purpureum* possesses two adjacent genes that are homologous to *matS* and *matT* (figs. S4 and S5). A clear *matD/matT* homolog is also evident in the more distantly related dictyostelid *Acytostelium subglobosum* (fig. S5). Adjacent to this gene is a small ORF very weakly similar to *matC/matS*, a possible *matS* ortholog in this species (fig. S6). *MatD* is distantly related to the Hap2-GCS1 family of gamete fusion proteins (20), supporting the idea that *MatD* and *MatT* are involved during fusion, although another *D. discoideum* gene, *hapA*, encodes a protein much more closely related to the canonical Hap2 group and is enriched in cells competent for mating (21).

The organization of the *Dictyostelium* mating-type locus does not closely resemble previously studied sex-determining regions (SOM text S5), although like them the *mat* locus must ultimately control a transcriptional cascade (21). Whether the

key genes directly regulate transcription or do so through downstream targets remains to be determined. One further role could be in controlling mitochondrial inheritance, which is uniparental in related Amoebozoans (22, 23).

We have identified the sex-determining locus from *D. discoideum*, a model organism and the best-studied member of the Amoebozoa. The master regulators of sex determination at this locus encode homologs of two small, apparently soluble proteins, which are unrelated to previously studied proteins. The genetic logic of the system allows one to speculate that one sex, mating type II, may have arisen after a fusion of the versions of the locus of the other two sexes. Understanding the mating-type locus may help to overcome longstanding difficulties in making use of sexual genetics in *Dictyostelium* (24).

References and Notes

1. I. Marín, B. S. Baker, *Science* **281**, 1990 (1998).
2. C. A. Smith *et al.*, *Nature* **461**, 267 (2009).
3. S. C. Lee, M. Ni, W. Li, C. Shertz, J. Heitman, *Microbiol. Mol. Biol. Rev.* **74**, 298 (2010).
4. M. Tanurdzic, J. A. Banks, *Plant Cell* **16** (suppl.), S61 (2004).
5. A. Martin *et al.*, *Nature* **461**, 1135 (2009).
6. J. C. Blaskovics, K. B. Raper, *Biol. Bull.* **113**, 58 (1957).
7. Y. Saga, H. Okada, K. Yanagisawa, *J. Cell Sci.* **60**, 157 (1983).
8. D. H. O'Day, *Can. J. Microbiol.* **25**, 1416 (1979).
9. M. F. Filosa, R. E. Dengler, *Dev. Biol.* **29**, 1 (1972).
10. M. A. Wallace, K. B. Raper, *J. Gen. Microbiol.* **113**, 327 (1979).
11. J. M. Flowers *et al.*, *PLoS Genet.* **6**, e1001013 (2010).
12. L. D. Hurst, *Proc. Biol. Sci.* **263**, 415 (1996).
13. G. W. Erdos, K. B. Raper, L. K. Vogen, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1828 (1973).
14. G. E. Robson, K. L. Williams, *Curr. Genet.* **1**, 229 (1980).
15. H. Urushihara, T. Muramoto, *Eur. J. Cell Biol.* **85**, 961 (2006).
16. G. E. Robson, K. L. Williams, *Genetics* **93**, 861 (1979).

17. L. Eichinger *et al.*, *Nature* **435**, 43 (2005).
18. G. Bloomfield, Y. Tanaka, J. Skelton, A. Ivens, R. R. Kay, *Genome Biol.* **9**, R75 (2008).
19. Materials and methods are available as supporting material on Science Online.
20. J. L. Wong, M. A. Johnson, *Trends Cell Biol.* **20**, 134 (2010).
21. T. Muramoto *et al.*, *Mech. Dev.* **120**, 965 (2003).
22. S. Kawano, R. W. Anderson, T. Nanba, T. Kuroiwa, *J. Gen. Microbiol.* **133**, 3175 (1987).
23. M. Mirfakhrai, Y. Tanaka, K. Yanagisawa, *Genetics* **124**, 607 (1990).
24. K. B. Raper, *The Dictyostelids* (Princeton Univ. Press, Princeton, NJ, 1984), chap. 9.
25. This work was supported by Wellcome Trust grant number 06724 and core funding from the Medical Research Council. We thank the Dicty Stock Center (www.dictybase.org/StockCenter/StockCenter.html), D. Francis, H. Hagiwara, and P. Schaap for providing strains; T. Feltwell, N. Nikolaidou-Katsaridou, and K. Jagels for technical assistance; and M. Fookes, C. Carret, K. Brooks, C. Pears, N. Barry, and the members and associate members of the *Dictyostelium* group at the Laboratory of Molecular Biology for valuable advice and discussions. A.I. is now at Fios Genomics, ETT, King's Buildings, Edinburgh EH9 3JL, UK. We are grateful to the U.S. Department of Energy Joint Genome Institute (www.jgi.doe.gov/) and co-workers, and to the *Actyostelium* Genome Consortium for making the *Dictyostelium purpureum* and *Acytostelium subglobosum* genome data available before publication. Microarray data are available under accession nos. E-TABM-394 and the array design under A-SGRP-3 at www.ebi.ac.uk/arrayexpress/, and DNA sequences have been deposited in EMBL-Bank under accessions nos. FN543120 to FN543124, FN994780, FR666792, and FR666793.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1533/DC1
Materials and Methods
SOM Text S1 to S5
Figs. S1 to S6
Tables S1 to S6
References

6 September 2010; accepted 28 October 2010
10.1126/science.1197423

Nebulin and N-WASP Cooperate to Cause IGF-1–Induced Sarcomeric Actin Filament Formation

Kazunori Takano,^{1,2} Haruko Watanabe-Takano,^{1,2} Shiro Suetsugu,^{2,3,4} Souichi Kurita,^{1*} Kazuya Tsujita,⁵ Sumiko Kimura,¹ Takashi Karatsu,⁶ Tadaomi Takenawa,^{2,5} Takeshi Endo^{1,2,†}

Insulin-like growth factor 1 (IGF-1) induces skeletal muscle maturation and enlargement (hypertrophy). These responses require protein synthesis and myofibril formation (myofibrillogenesis). However, the signaling mechanisms of myofibrillogenesis remain obscure. We found that IGF-1–induced phosphatidylinositol 3-kinase–Akt signaling formed a complex of nebulin and N-WASP at the Z bands of myofibrils by interfering with glycogen synthase kinase-3 β in mice. Although N-WASP is known to be an activator of the Arp2/3 complex to form branched actin filaments, the nebulin–N-WASP complex caused actin nucleation for unbranched actin filament formation from the Z bands without the Arp2/3 complex. Furthermore, N-WASP was required for IGF-1–induced muscle hypertrophy. These findings present the mechanisms of IGF-1–induced actin filament formation in myofibrillogenesis required for muscle maturation and hypertrophy and a mechanism of actin nucleation.

IGF-1–induced muscle maturation and hypertrophy necessitate not only protein synthesis but also myofibrillogenesis. IGF-1 causes pro-

tein synthesis by activating phosphatidylinositol 3-kinase (PI3K)–Akt signaling (1, 2). In contrast, the signaling mechanisms of myofibrillogenesis

are unclear. Although the proteins inducing actin filament formation in nonmuscle cells have been elucidated (3–7), those responsible for actin filament formation from the Z bands of myofibrils remain unknown. Thus, we asked whether N-WASP, which causes rapid actin polymerization by activating the Arp2/3 complex in nonmuscle cells, is involved in myofibrillar actin filament formation.

¹Department of Biology, Graduate School of Science, Chiba University, 1-33 Yayoicho, Inageku, Chiba 263-8522, Japan.

²Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), Chiyodaku, Tokyo 102-0075, Japan. ³Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo 113-0032, Japan. ⁴Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency (JST), Chiyodaku, Tokyo 102-0075, Japan. ⁵Division of Lipid Biochemistry, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan. ⁶Department of Applied Chemistry and Biotechnology, Graduate School of Engineering, Chiba University, 1-33 Yayoicho, Inageku, Chiba 263-8522, Japan.

*Present address: Division of Molecular and Cellular Biology, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan.

†To whom correspondence should be addressed. E-mail: t.endo@faculty.chiba-u.jp

N-WASP was localized to the Z bands in mature skeletal muscle and became confined to the Z bands during muscle regeneration (Fig.

1A and fig. S1, A and B). Then we examined the location of N-WASP in the skeletal muscle of IGF-1-administered mice, which were prefasted

for 48 hours. Although N-WASP was diffusely distributed throughout the myofibers in the fasted muscle, it was mobilized to the Z bands within 30

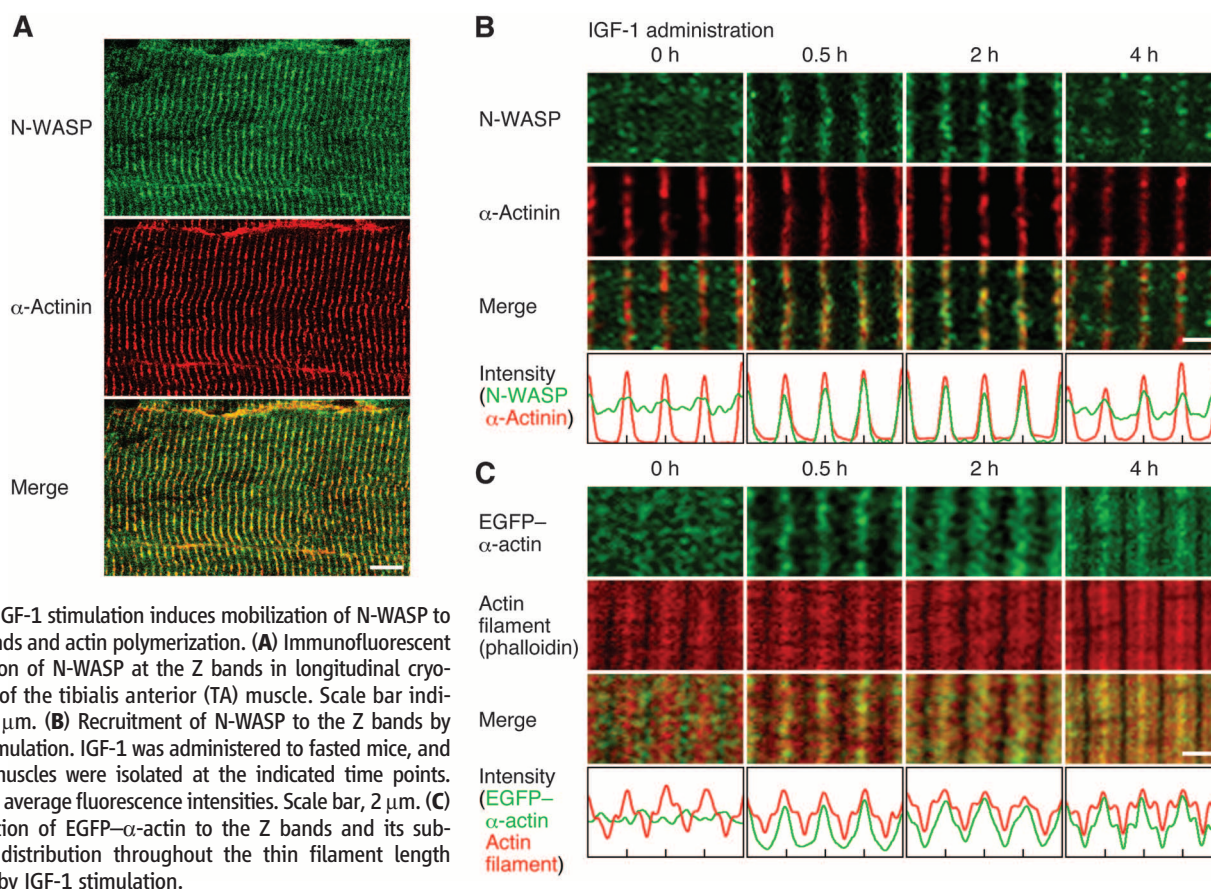
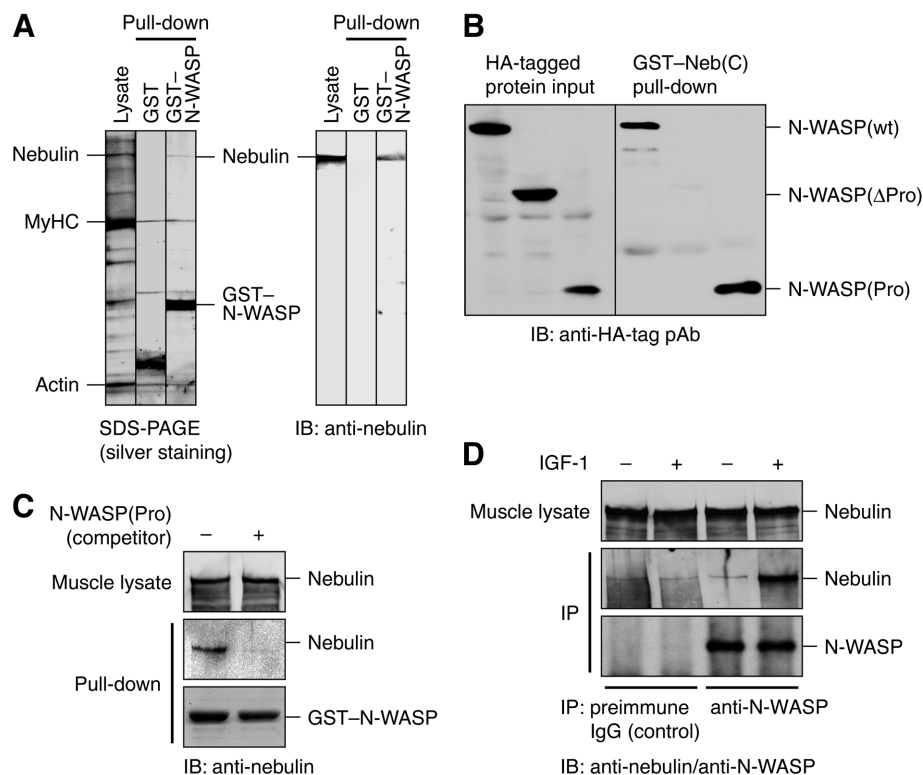


Fig. 1. IGF-1 stimulation induces mobilization of N-WASP to the Z bands and actin polymerization. **(A)** Immunofluorescent localization of N-WASP at the Z bands in longitudinal cryosections of the tibialis anterior (TA) muscle. Scale bar indicates 10 μ m. **(B)** Recruitment of N-WASP to the Z bands by IGF-1 stimulation. IGF-1 was administered to fasted mice, and the TA muscles were isolated at the indicated time points. Intensity, average fluorescence intensities. Scale bar, 2 μ m. **(C)** Mobilization of EGFP- α -actin to the Z bands and its subsequent distribution throughout the thin filament length induced by IGF-1 stimulation.

Fig. 2. N-WASP binds to the nebulin C-terminal region by IGF-1 stimulation. **(A)** Binding of nebulin in skeletal muscle lysates to N-WASP, detected by a GST-N-WASP pull-down assay. Shown are SDS-polyacrylamide gel electrophoresis patterns and corresponding immunoblots (IB) with an antibody against nebulin (anti-nebulin). MyHC, myosin heavy chain. **(B)** Binding of N-WASP and N-WASP(Pro) to Neb(C), detected by a GST-Neb(C) pull-down assay. Hemagglutinin (HA)-tagged N-WASP and its mutants were expressed in COS-1 cells. **(C)** Binding of nebulin in muscle lysates to N-WASP, detected by a GST-N-WASP pull-down assay. N-WASP(Pro) was added as a binding competitor. **(D)** Induction of the binding of N-WASP to nebulin by IGF-1 stimulation, detected by coimmunoprecipitation with N-WASP. Muscle lysates prepared 60 min after IGF-1 administration to fasted mice were used for immunoprecipitation (IP).



min after IGF-1 stimulation (Fig. 1B). N-WASP was associated with the Z bands and colocalized with α -actinin by 2 hours after the stimulation, but became diffusely distributed again by 4 hours. α -actinin was restricted to the Z bands in both the fasted and the stimulated muscles, indicating that the Z bands remained intact during these periods.

A plasmid encoding enhanced green fluorescent protein (EGFP)-tagged α -actin was introduced into the fasted muscle. Although the EGFP- α -actin was diffusely distributed in the fasted muscle myofibers in the absence of stimulation, it was mobilized to the Z bands within 30 min after IGF-1 stimulation (Fig. 1C). It was distributed gradually in broader stripes away from the Z bands and eventually almost throughout the entire length of the thin filaments, which were detected by phalloidin staining by 4 hours. Thus, IGF-1 signaling induces the recruitment of N-WASP to the Z bands, and N-WASP may be involved in the formation of actin thin filaments from the Z bands without the Arp2/3 complex (fig. S1, C and D).

To identify the protein that recruits N-WASP to the Z bands in response to IGF-1 stimulation, we searched for N-WASP-binding protein(s) associated with the Z bands. In a binding (pull-down)

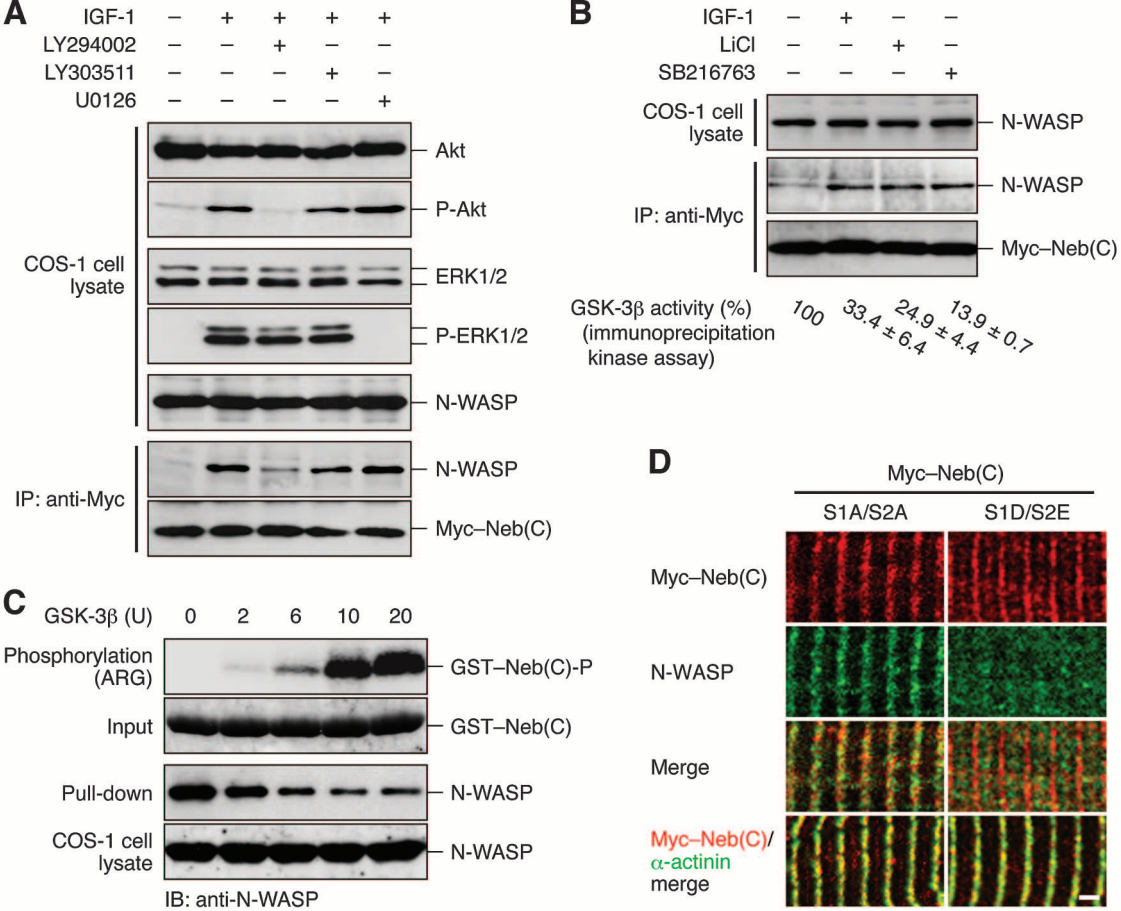
assay with glutathione *S*-transferase (GST)-tagged N-WASP, nebulin bound to N-WASP (Fig. 2A). Nebulin is a giant (500 to 900 kD) sarcomeric protein spanning almost the entire length of thin filaments (8–11). Its C-terminal region, which is associated with the Z band, contains the Src homology 3 (SH3) domain (fig. S2, A and B). N-WASP has a proline (Pro)-rich region (fig. S2C), where several SH3 domain-containing proteins, such as Grb2, bind to activate N-WASP by inducing the open conformation (3). We thus examined whether the N-WASP Pro-rich region [N-WASP(Pro)] bound to the SH3 domain-containing C-terminal region of nebulin [Neb(C)]. N-WASP and N-WASP(Pro) but not Pro-rich region-deleted N-WASP [N-WASP(Δ Pro)] bound to Neb(C) (Fig. 2B and fig. S3, A and B). Furthermore, N-WASP(Pro) competed for the binding of GST-N-WASP and nebulin in skeletal muscle lysates (Fig. 2C). Accordingly, N-WASP binds through its Pro-rich region to the SH3 domain in the C-terminal region of nebulin in vitro. When N-WASP was immunoprecipitated with the antibody against N-WASP (anti-N-WASP) from muscle lysates of fasted mice, nebulin was scarcely coprecipitated with N-WASP (Fig. 2D). Administration of IGF-1 to the mice, however, resulted in coprecipitation of nebulin with N-WASP

(Fig. 2D and fig. S3C). Therefore, IGF-1 stimulation allows N-WASP to bind to nebulin in vivo, and N-WASP is recruited to the Z bands through the binding to nebulin.

Next, we addressed how IGF-1 signaling induced the binding of N-WASP to nebulin. IGF-1 stimulation activates two signaling pathways, PI3K-Akt signaling and the Ras-extracellular signal-regulated kinase (ERK) pathway. When COS-1 cells were stimulated with IGF-1, activating phosphorylation of both Akt and ERK occurred (Fig. 3A). The phosphorylation of Akt and ERK was prevented by treatment with the PI3K inhibitor LY294002 and the mitogen-activated protein kinase kinase (MEK) inhibitor U0126, respectively. Analyses with these inhibitors indicated that PI3K-Akt signaling but not the Ras-ERK pathway participated in the binding of N-WASP to Myc-Neb(C) (Fig. 3A and fig. S4, A and B).

One of the target proteins of Akt is glycogen synthase kinase-3 β (GSK-3 β), and phosphorylation by Akt inhibits its kinase activity (12). When COS-1 cells transfected with Myc-Neb(C) were treated with a GSK-3 β inhibitor, LiCl or SB216763, N-WASP was co-precipitated with Myc-Neb(C), even without IGF-1 stimulation (Fig. 3B). Moreover, GSK-3 β phosphorylated GST-Neb(C) dose-

Fig. 3. IGF-1-induced PI3K-Akt signaling causes nebulin–N-WASP interaction by suppressing GSK-3 β . (A) Induction of nebulin–N-WASP interaction by PI3K-Akt signaling. Myc-Neb(C)-transfected COS-1 cells were stimulated with IGF-1 in the presence of the PI3K inhibitor LY294002, its noninhibitory analog LY303511, or the MEK inhibitor U0126. Activating phosphorylation of Akt and ERK1/2 was detected with phospho-antibodies. The binding of N-WASP to Myc-Neb(C) was detected by coimmunoprecipitation with the monoclonal antibody (mAb) to Myc (anti-Myc). (B) Facilitation of the binding of N-WASP to Neb(C) by inhibiting GSK-3 β . Myc-Neb(C)-transfected COS-1 cells were stimulated with IGF-1 or treated with GSK-3 β inhibitors, LiCl and SB216763. The binding of N-WASP to Myc-Neb(C) was detected by coimmunoprecipitation with anti-Myc. (C) Abrogation of the binding of N-WASP to Neb(C) phosphorylated by GSK-3 β , detected by a GST-Neb(C) pull-down assay. Phosphorylation of GST-Neb(C) was analyzed by autoradiography (ARG). U, units. (D) Binding of N-WASP to unphosphorylatable Neb(C) but not to pseudo-phosphorylated Neb(C) expressed in the muscle. The TA



muscles were transfected with Myc-Neb(C)(S1A/S2A) or Myc-Neb(C)(S1D/S2E). The localization of Myc-Neb(C) and N-WASP was detected in the fasted mouse muscle. Scale bar, 2 μ m.

independently in vitro (Fig. 3C and fig S4C). Unphosphorylated Neb(C) strongly bound N-WASP, but the binding level was reduced according to the degree of phosphorylation. Thus, the phosphorylation of Neb(C) by GSK-3 β inhibits the interaction of nebulin with N-WASP. Indeed, there are two consensus serine residues (Ser1 and Ser2) phosphorylatable by GSK-3 β in the Ser-rich region adjacent to the SH3 domain of nebulin (fig. S2B). To determine whether GSK-3 β phosphorylates them, we mutated the Ser (S) to Ala (A) [Neb(C)(S1A) and Neb(C)(S1A/S2A)]. GSK-3 β efficiently phosphorylated GST-tagged wild-type Neb(C) in vitro, whereas it phosphorylated Neb(C)(S1A) less efficiently and did not phosphorylate Neb(C)(S1A/S2A) (fig. S4, D to G).

Administration of IGF-1 to fasted mice caused the activating phosphorylation of Akt and the inhibiting phosphorylation of GSK-3 β by Akt in skeletal muscle (fig. S5A). Both Ser¹ and Ser² in nebulin were phosphorylated at high levels in the fasted mouse muscle, but IGF-1 stimulation prominently reduced their phosphorylation lev-

els. Endogenous N-WASP was located to the Z bands when Myc-Neb(C)(S1A/S2A) was expressed, whereas it was diffusely distributed when Myc-Neb(C)(S1D/S2E) (where D indicates Asp and E indicates Glu) was expressed (Fig. 3D). These results and those in fig. S5, B to F, imply that IGF-1–induced PI3K–Akt signaling suppresses GSK-3 β by phosphorylation and consequently prevents the phosphorylation of Ser1 and Ser2 in nebulin by GSK-3 β on the Z bands. Unphosphorylated nebulin but not phosphorylated nebulin can bind N-WASP and recruit it to the Z bands.

Because the Arp2/3 complex was not associated with myofibrils (fig. S1, C and D), we investigated the possibility that N-WASP induces myofibrillar actin filament formation in the absence of the Arp2/3 complex. N-WASP has two WASP homology 2 (WH2) domains (fig. S2C), which are actin monomer-binding motifs present in several actin nucleators and elongators (3, 5–7, 13). On the other hand, nebulin contains 185 tandem modules (fig. S2A), which contain an actin monomer-binding motif in each repeat (8, 14). We thus

examined whether the two WH2 domains of N-WASP cooperate with module 182 (M182) to M185 of nebulin to induce actin nucleation or elongation (fig. S6, A and B). In a fluorometric actin polymerization assay in the absence of N-WASP, Neb(C)2M [Neb(C) with M184 and M185] (fig. S6A) facilitated actin polymerization only weakly, Neb(C)3M facilitated moderately, and Neb(C)4M more efficiently (Fig. 4A and fig. S6C). In the presence of N-WASP, all of the Neb(C)Ms polymerized actin much more efficiently than in the absence of N-WASP. In contrast, neither N-WASP(Δ Pro), which cannot bind to Neb(C), nor N-WASP(Δ WCA), which lacks the WCA region involved in actin monomer recruitment, enhanced actin polymerization by Neb(C)Ms (fig. S6D). Accordingly, Neb(C)Ms and N-WASP cooperate to polymerize actin.

The number of unbranched actin filament seeds increased proportionally to the concentration of Neb(C)Ms in the presence of N-WASP (movies S1 to S10 and fig. S7, A and B). Barbed end formation and nucleation rates by Neb(C)Ms were facilitated

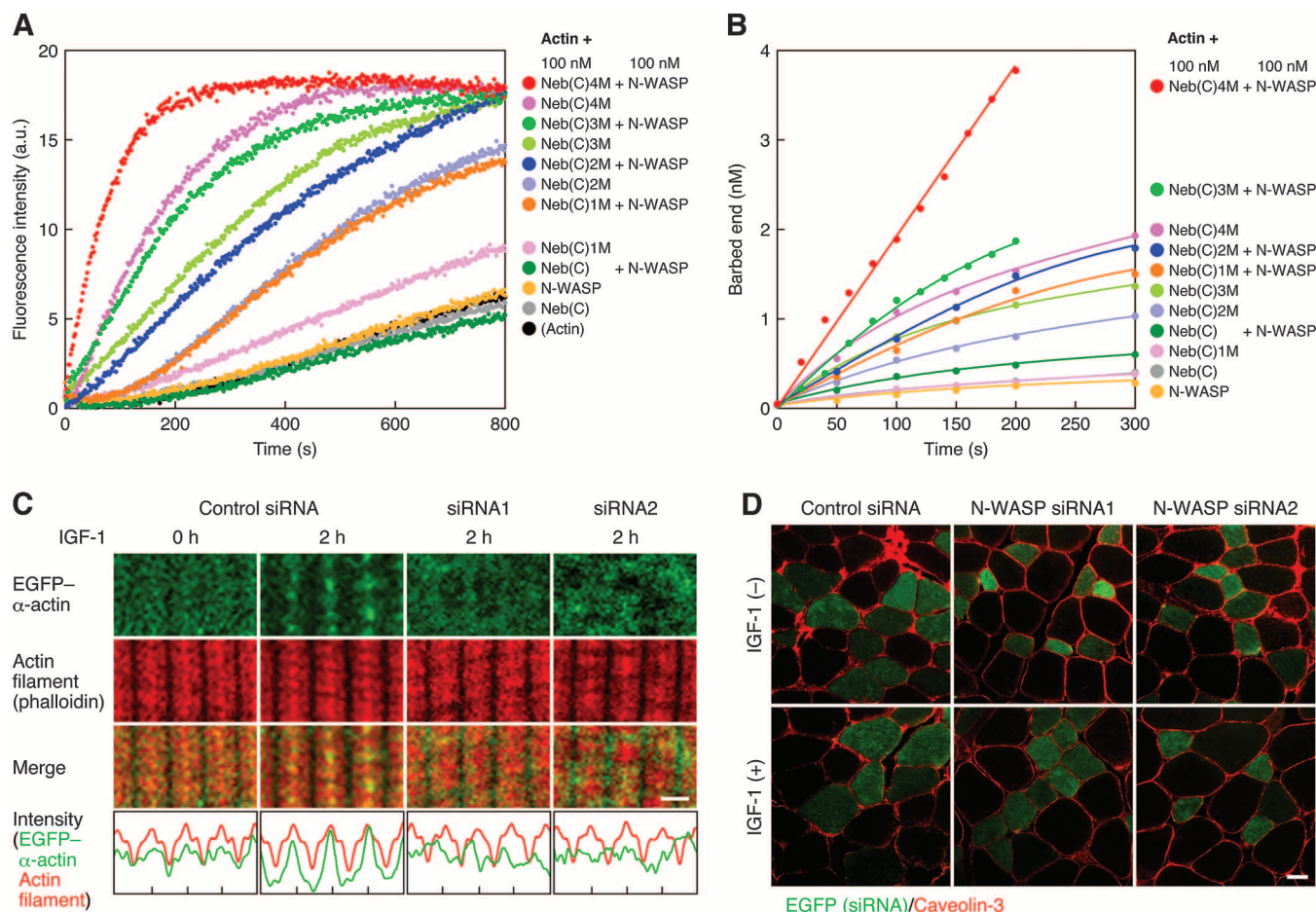


Fig. 4. The nebulin–N-WASP complex induces actin filament formation, and N-WASP is essential for muscle hypertrophy. **(A)** Promotion of actin polymerization by Neb(C)Ms with N-WASP, detected by a pyrene-actin polymerization assay. a.u., arbitrary units. **(B)** Facilitation of barbed-end formation by Neb(C)Ms with N-WASP, calculated from the results of total internal reflection fluorescence (TIRF) microscopy. **(C)** Suppression of α -actin incorporation into the Z bands and thin filaments by N-WASP RNAi. Muscles were transfected

with the siRNA expression vectors and EGFP– α -actin. IGF-1 was administered to fasted mice, and the muscles were isolated at 2 hours. Scale bar, 2 μ m. **(D)** Suppression of natural and IGF-1–induced muscle hypertrophy by N-WASP RNAi. Muscles were transfected with the siRNA expression vectors and pEGFP-C1 vector to monitor the siRNA-expressing myofibers. IGF-1 was administered every 2 days for 14 days to mice (from 35 days to 49 days old). Cross sections of the muscle are shown. Scale bar, 20 μ m.

by N-WASP (Fig. 4B and fig. S7, C to E). The results suggest that nebulin modules and the N-WASP WH2 domains cooperate to nucleate an unbranched actin filament. Then the actin filament might elongate along the nebulin modules from the Z band toward the center of a sarcomere (fig. S8A).

We assessed the requirement of N-WASP for IGF-1-induced actin filament formation in myofibrils and muscle hypertrophy by RNA interference (RNAi) (fig. S9). EGFP- α -actin coexpressed with control small interfering RNA (siRNA) in the fasted mouse muscle was diffusely distributed without IGF-1 stimulation but located to the Z bands and thin filaments within 2 hours after the stimulation (Fig. 4C and fig. S9D). In contrast, EGFP- α -actin coexpressed with siRNA1 or 2 (fig. S11A) remained diffusely distributed after the stimulation. Therefore, N-WASP is indispensable for the recruitment of α -actin to the Z bands and for myofibrillar actin filament formation.

IGF-1 administration to mice caused muscle hypertrophy owing to the increase in myofiber volume. The expression of siRNA1 or 2 reduced the cross-sectional area of the myofibers regardless of IGF-1 administration (Fig. 4D and figs. S10 and S11). Thus, N-WASP plays essential roles in both age-dependent natural hypertrophy and administered IGF-1-induced hypertrophy. N-

WASP seems to participate in myofiber hypertrophy by inducing myofibrillar actin filament formation through the nebulin-N-WASP complex. This notion is consistent with the observation that *Neb*-deficient mice develop a muscle atrophy-like phenotype (15, 16).

We elucidated the signaling of IGF-1-induced myofibrillar actin filament formation from the Z bands (fig. S8B) and a mechanism of actin nucleation [supporting online material (SOM) text]. The Neb-N-WASP complex formed by the signaling can explain actin filament formation arising from the Z bands. These findings may provide insights into the mechanisms of muscular diseases, such as nemaline myopathy, caused by *NEB* gene mutations (17). The actin filament formation together with myosin filament assembly, which might also induced by IGF-1 signaling, results in myofibrillogenesis required for muscle maturation and hypertrophy.

References and Notes

1. D. J. Glass, *Int. J. Biochem. Cell Biol.* **37**, 1974 (2005).
2. F. Mourikoti, N. Rosenthal, *Trends Immunol.* **26**, 535 (2005).
3. T. Takenawa, S. Suetsugu, *Nat. Rev. Mol. Cell Biol.* **8**, 37 (2007).
4. T. D. Pollard, G. G. Borisov, *Cell* **112**, 453 (2003).
5. M. A. Chesaron, B. L. Goode, *Curr. Opin. Cell Biol.* **21**, 28 (2009).

6. B. Qualmann, M. M. Kessels, *Trends Cell Biol.* **19**, 276 (2009).
7. R. Dominguez, *Crit. Rev. Biochem. Mol. Biol.* **44**, 351 (2009).
8. S. Labeit, B. Kolmerer, *J. Mol. Biol.* **248**, 308 (1995).
9. K. Ojima *et al.*, *J. Cell Biol.* **150**, 553 (2000).
10. A. S. McElhinny, S. T. Kazmierski, S. Labeit, C. C. Gregorio, *Trends Cardiovasc. Med.* **13**, 195 (2003).
11. A. Kontrogianni-Konstantopoulos, M. A. Ackermann, A. L. Bowman, S. V. Yap, R. J. Bloch, *Physiol. Rev.* **89**, 1217 (2009).
12. B. D. Manning, L. C. Cantley, *Cell* **129**, 1261 (2007).
13. E. Paunola, P. K. Mattila, P. Lappalainen, *FEBS Lett.* **513**, 92 (2002).
14. M. Pfuhl, S. J. Winder, M. A. Castiglione Morelli, S. Labeit, A. Pastore, *J. Mol. Biol.* **257**, 367 (1996).
15. C. C. Witt *et al.*, *EMBO J.* **25**, 3843 (2006).
16. M.-L. Bang *et al.*, *J. Cell Biol.* **173**, 905 (2006).
17. D. Sanoudou, A. H. Beggs, *Trends Mol. Med.* **7**, 362 (2001).
18. We thank N. Watanabe and T. Itoh for comments. This work was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and by the Research Grants (17A-10 and 20B-13) for Nervous and Mental Disorders from the Ministry of Health, Labor, and Welfare of Japan.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1536/DC1
Materials and Methods

SOM Text

Figs. S1 to S11

References

Movies S1 to S10

14 September 2010; accepted 1 November 2010

10.1126/science.1197767

Genome Evolution Following Host Jumps in the Irish Potato Famine Pathogen Lineage

Sylvain Raffaele,^{1*} Rhys A. Farrer,^{1,*†} Liliana M. Cano,^{1,*} David J. Studholme,^{1,‡} Daniel MacLean,¹ Marco Thines,^{1,2,3} Rays H. Y. Jiang,⁴ Michael C. Zody,⁴ Sridhara G. Kunjeti,⁵ Nicole M. Donofrio,⁵ Blake C. Meyers,⁵ Chad Nusbaum,⁴ Sophien Kamoun^{1§}

Many plant pathogens, including those in the lineage of the Irish potato famine organism *Phytophthora infestans*, evolve by host jumps followed by specialization. However, how host jumps affect genome evolution remains largely unknown. To determine the patterns of sequence variation in the *P. infestans* lineage, we resequenced six genomes of four sister species. This revealed uneven evolutionary rates across genomes with genes in repeat-rich regions showing higher rates of structural polymorphisms and positive selection. These loci are enriched in genes induced in planta, implicating host adaptation in genome evolution. Unexpectedly, genes involved in epigenetic processes formed another class of rapidly evolving residents of the gene-sparse regions. These results demonstrate that dynamic repeat-rich genome compartments underpin accelerated gene evolution following host jumps in this pathogen lineage.

Phytophthora infestans is an economically important specialized pathogen that causes the destructive late blight disease on *Solanum* plants, including potato and tomato. In central Mexico, *P. infestans* naturally co-occurs with two extremely closely related species, *Phytophthora ipomoeae* and *Phytophthora mirabilis*, that specifically infect plants as diverse as morning glory (*Ipomoea longipedunculata*) and four-o'clock (*Mirabilis jalapa*), respectively. Elsewhere in North America, a fourth related species, *Phytophthora phaseoli*, is a pathogen of lima beans (*Phaseolus lunatus*). Altogether these four *Phytophthora* spe-

cies form a very tight clade of pathogen species that share ~99.9% identity in their ribosomal DNA internal transcribed spacer regions (1). Phylogenetic inferences clearly indicate that species in this *Phytophthora* clade 1c [nomenclature of (2)] evolved through host jumps followed by adaptive specialization on plants belonging to four different botanical families (2, 3). Adaptation to these host plants most likely involves mutations in the hundreds of disease effector genes that populate gene-poor and repeat-rich regions of the 240-megabase pair genome of *P. infestans* (4). However, comparative genome analyses of specialized sister species

of plant pathogens have not been reported, and the full extent to which host adaptation affects genome evolution remains unknown.

To determine patterns of sequence variation in a phylogenetically defined species cluster of host-specific plant pathogens, we generated Illumina reads for six genomes representing the four clade 1c species. We included the previously sequenced *P. infestans* strain T30-4 (4) to optimize bioinformatic parameters (figs. S1 to S3) (5). To estimate gene copy number variation (CNV) in the five resequenced genomes relative to T30-4, we used average read depth per gene and GC content correction (5) (fig. S4). After GC content correction (6), average read depth provided a good estimate of gene copy number in T30-4 (fig. S5). In the other genomes, we detected 3975 CNV events in coding genes, among which there are 1046 deletion events (Fig. 1).

¹The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, UK. ²Biodiversity and Climate Research Centre BiK-F, Senckenberganlage 25, D-60325 Frankfurt (Main), Germany. ³Department of Biological Sciences, Institute of Ecology, Evolution and Diversity, Johann Wolfgang Goethe University, Siesmayerstraße 70, D-60323 Frankfurt (Main), Germany. ⁴Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA 02141, USA. ⁵University of Delaware, Newark, DE 19716, USA.

*These authors contributed equally to this work.

†Present address: Imperial College London, London SW7 2AZ, UK.

‡Present address: Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QD, UK.

§To whom correspondence should be addressed. E-mail: sophien.kamoun@tsl.ac.uk

by N-WASP (Fig. 4B and fig. S7, C to E). The results suggest that nebulin modules and the N-WASP WH2 domains cooperate to nucleate an unbranched actin filament. Then the actin filament might elongate along the nebulin modules from the Z band toward the center of a sarcomere (fig. S8A).

We assessed the requirement of N-WASP for IGF-1-induced actin filament formation in myofibrils and muscle hypertrophy by RNA interference (RNAi) (fig. S9). EGFP- α -actin coexpressed with control small interfering RNA (siRNA) in the fasted mouse muscle was diffusely distributed without IGF-1 stimulation but located to the Z bands and thin filaments within 2 hours after the stimulation (Fig. 4C and fig. S9D). In contrast, EGFP- α -actin coexpressed with siRNA1 or 2 (fig. S11A) remained diffusely distributed after the stimulation. Therefore, N-WASP is indispensable for the recruitment of α -actin to the Z bands and for myofibrillar actin filament formation.

IGF-1 administration to mice caused muscle hypertrophy owing to the increase in myofiber volume. The expression of siRNA1 or 2 reduced the cross-sectional area of the myofibers regardless of IGF-1 administration (Fig. 4D and figs. S10 and S11). Thus, N-WASP plays essential roles in both age-dependent natural hypertrophy and administered IGF-1-induced hypertrophy. N-

WASP seems to participate in myofiber hypertrophy by inducing myofibrillar actin filament formation through the nebulin-N-WASP complex. This notion is consistent with the observation that *Neb*-deficient mice develop a muscle atrophy-like phenotype (15, 16).

We elucidated the signaling of IGF-1-induced myofibrillar actin filament formation from the Z bands (fig. S8B) and a mechanism of actin nucleation [supporting online material (SOM) text]. The Neb-N-WASP complex formed by the signaling can explain actin filament formation arising from the Z bands. These findings may provide insights into the mechanisms of muscular diseases, such as nemaline myopathy, caused by *NEB* gene mutations (17). The actin filament formation together with myosin filament assembly, which might also induced by IGF-1 signaling, results in myofibrillogenesis required for muscle maturation and hypertrophy.

References and Notes

1. D. J. Glass, *Int. J. Biochem. Cell Biol.* **37**, 1974 (2005).
2. F. Mourikoti, N. Rosenthal, *Trends Immunol.* **26**, 535 (2005).
3. T. Takenawa, S. Suetsugu, *Nat. Rev. Mol. Cell Biol.* **8**, 37 (2007).
4. T. D. Pollard, G. G. Borisov, *Cell* **112**, 453 (2003).
5. M. A. Chesaron, B. L. Goode, *Curr. Opin. Cell Biol.* **21**, 28 (2009).

6. B. Qualmann, M. M. Kessels, *Trends Cell Biol.* **19**, 276 (2009).
7. R. Dominguez, *Crit. Rev. Biochem. Mol. Biol.* **44**, 351 (2009).
8. S. Labeit, B. Kolmerer, *J. Mol. Biol.* **248**, 308 (1995).
9. K. Ojima *et al.*, *J. Cell Biol.* **150**, 553 (2000).
10. A. S. McElhinny, S. T. Kazmierski, S. Labeit, C. C. Gregorio, *Trends Cardiovasc. Med.* **13**, 195 (2003).
11. A. Kontrogianni-Konstantopoulos, M. A. Ackermann, A. L. Bowman, S. V. Yap, R. J. Bloch, *Physiol. Rev.* **89**, 1217 (2009).
12. B. D. Manning, L. C. Cantley, *Cell* **129**, 1261 (2007).
13. E. Paunola, P. K. Mattila, P. Lappalainen, *FEBS Lett.* **513**, 92 (2002).
14. M. Pfuhl, S. J. Winder, M. A. Castiglione Morelli, S. Labeit, A. Pastore, *J. Mol. Biol.* **257**, 367 (1996).
15. C. C. Witt *et al.*, *EMBO J.* **25**, 3843 (2006).
16. M.-L. Bang *et al.*, *J. Cell Biol.* **173**, 905 (2006).
17. D. Sanoudou, A. H. Beggs, *Trends Mol. Med.* **7**, 362 (2001).
18. We thank N. Watanabe and T. Itoh for comments. This work was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and by the Research Grants (17A-10 and 20B-13) for Nervous and Mental Disorders from the Ministry of Health, Labor, and Welfare of Japan.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1536/DC1
Materials and Methods

SOM Text

Figs. S1 to S11

References

Movies S1 to S10

14 September 2010; accepted 1 November 2010

10.1126/science.1197767

Genome Evolution Following Host Jumps in the Irish Potato Famine Pathogen Lineage

Sylvain Raffaele,^{1*} Rhys A. Farrer,^{1,*†} Liliana M. Cano,^{1*} David J. Studholme,^{1,‡} Daniel MacLean,¹ Marco Thines,^{1,2,3} Rays H. Y. Jiang,⁴ Michael C. Zody,⁴ Sridhara G. Kunjeti,⁵ Nicole M. Donofrio,⁵ Blake C. Meyers,⁵ Chad Nusbaum,⁴ Sophien Kamoun^{1§}

Many plant pathogens, including those in the lineage of the Irish potato famine organism *Phytophthora infestans*, evolve by host jumps followed by specialization. However, how host jumps affect genome evolution remains largely unknown. To determine the patterns of sequence variation in the *P. infestans* lineage, we resequenced six genomes of four sister species. This revealed uneven evolutionary rates across genomes with genes in repeat-rich regions showing higher rates of structural polymorphisms and positive selection. These loci are enriched in genes induced in planta, implicating host adaptation in genome evolution. Unexpectedly, genes involved in epigenetic processes formed another class of rapidly evolving residents of the gene-sparse regions. These results demonstrate that dynamic repeat-rich genome compartments underpin accelerated gene evolution following host jumps in this pathogen lineage.

Phytophthora infestans is an economically important specialized pathogen that causes the destructive late blight disease on *Solanum* plants, including potato and tomato. In central Mexico, *P. infestans* naturally co-occurs with two extremely closely related species, *Phytophthora ipomoeae* and *Phytophthora mirabilis*, that specifically infect plants as diverse as morning glory (*Ipomoea longipedunculata*) and four-o'clock (*Mirabilis jalapa*), respectively. Elsewhere in North America, a fourth related species, *Phytophthora phaseoli*, is a pathogen of lima beans (*Phaseolus lunatus*). Altogether these four *Phytophthora* spe-

cies form a very tight clade of pathogen species that share ~99.9% identity in their ribosomal DNA internal transcribed spacer regions (1). Phylogenetic inferences clearly indicate that species in this *Phytophthora* clade 1c [nomenclature of (2)] evolved through host jumps followed by adaptive specialization on plants belonging to four different botanical families (2, 3). Adaptation to these host plants most likely involves mutations in the hundreds of disease effector genes that populate gene-poor and repeat-rich regions of the 240-megabase pair genome of *P. infestans* (4). However, comparative genome analyses of specialized sister species

of plant pathogens have not been reported, and the full extent to which host adaptation affects genome evolution remains unknown.

To determine patterns of sequence variation in a phylogenetically defined species cluster of host-specific plant pathogens, we generated Illumina reads for six genomes representing the four clade 1c species. We included the previously sequenced *P. infestans* strain T30-4 (4) to optimize bioinformatic parameters (figs. S1 to S3) (5). To estimate gene copy number variation (CNV) in the five resequenced genomes relative to T30-4, we used average read depth per gene and GC content correction (5) (fig. S4). After GC content correction (6), average read depth provided a good estimate of gene copy number in T30-4 (fig. S5). In the other genomes, we detected 3975 CNV events in coding genes, among which there are 1046 deletion events (Fig. 1).

¹The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, UK. ²Biodiversity and Climate Research Centre BiK-F, Senckenberganlage 25, D-60325 Frankfurt (Main), Germany. ³Department of Biological Sciences, Institute of Ecology, Evolution and Diversity, Johann Wolfgang Goethe University, Siesmayerstraße 70, D-60323 Frankfurt (Main), Germany. ⁴Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA 02141, USA. ⁵University of Delaware, Newark, DE 19716, USA.

*These authors contributed equally to this work.

†Present address: Imperial College London, London SW7 2AZ, UK.

‡Present address: Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QD, UK.

§To whom correspondence should be addressed. E-mail: sophien.kamoun@tsl.ac.uk

In total, we identified 746,744 nonredundant coding sequence single-nucleotide polymorphisms (SNPs) in the resequenced strains (Fig. 1). We calculated rates of synonymous (dS) and nonsynonymous (dN) substitutions for every gene (5, 7). Average dS divergence rates relative to *P. infestans*

T30-4 were consistent with previously reported species phylogeny (Fig. 1) (2). We detected a total of 2572 genes (14.2% of the whole genome) with dN/dS ratios >1 indicative of positive selection in the clade 1c strains, with the highest number in *P. mirabilis* (1004 genes) (fig. S6). A high proportion of genes annotated as effector genes show signatures of positive selection (300 out of 796) (fig. S6). This supports previous observations that effector genes are under strong positive selection in oomycetes (8–10).
Haas *et al.* (4) reported that the *P. infestans* genome experienced a repeat-driven expansion relative to distantly related *Phytophthora* spp. and shows an unusual discontinuous distribution of gene density. Disease effector genes localize to expanded, repeat-rich and gene-sparse regions of the genome, in contrast to core ortholog genes, which occupy repeat-poor and gene-dense regions (4). We exploited our sequence data to determine the extent to which genomic regions with distinct architecture evolved at different rates. We used statistical tests (table S1) and random sampling

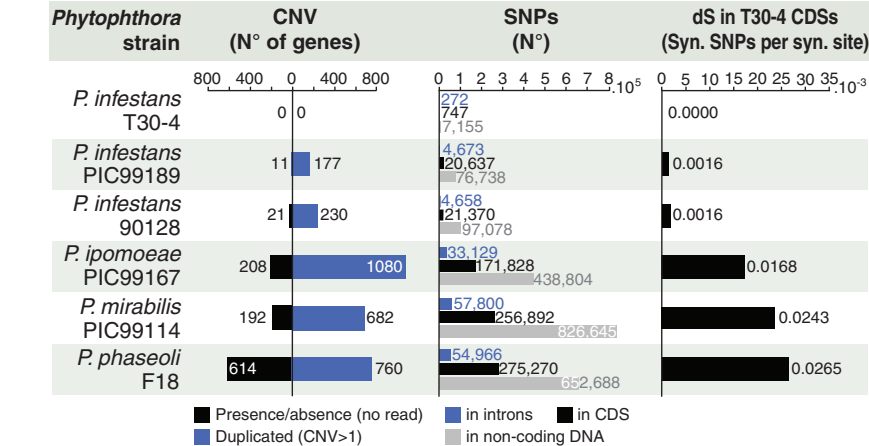
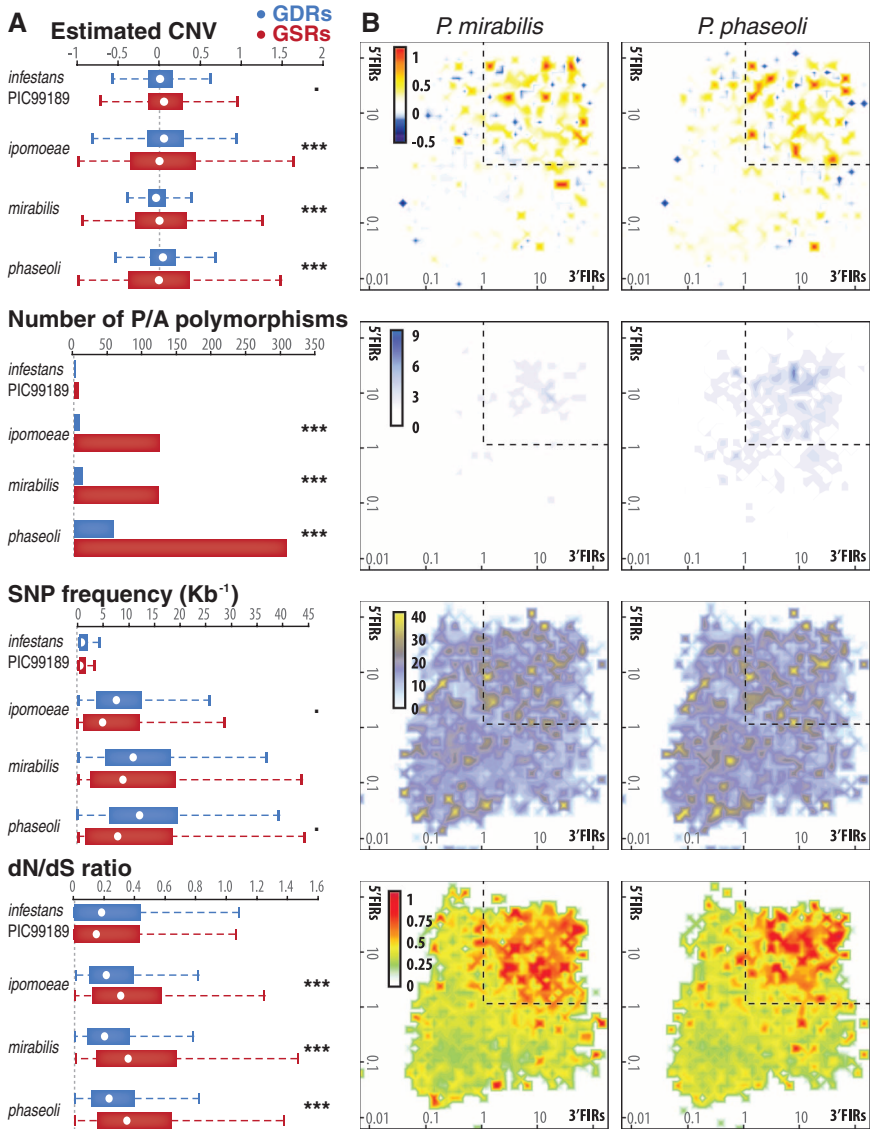


Fig. 1. Summary of genome sequences obtained for *Phytophthora* clade 1c species. Six strains representing four species were analyzed. *P. infestans* T30-4 previously sequenced by Haas *et al.* (4) was included for quality control. CDS, coding sequence; CNV, copy number variation; SNP, single-nucleotide polymorphism; syn., synonymous.

Fig. 2. The two-speed genome of *P. infestans*. (A) Distribution of copy number variation (CNV), presence/absence (P/A) and single-nucleotide polymorphisms (SNP), and dN/dS in genes from gene-dense regions (GDRs) and gene-sparse regions (GSRs). Statistical significance was assessed by unpaired *t* test assuming unequal variance (CNV, dN/dS); assuming equal variance (SNP frequency); or by Fisher's exact test (P/A) (•*P* < 0.1; ****P* < 10^{−4}). Whiskers show first value outside 1.5 times the interquartile range. (B) Distribution of polymorphism in *P. mirabilis* and *P. phaseoli* according to local gene density (measured as length of 5' and 3' flanking intergenic regions, FIRs). The number of genes (P/A polymorphisms) or average values (CNV, SNP, dN/dS) associated with genes in each bin are shown as a color-coded heat map.



(table S2) to determine the significance of differences in CNV, presence/absence polymorphisms, SNP frequency, and dN/dS values in genes located in gene-dense versus gene-sparse regions (5) (fig. S7 and table S3). Although averages of gene copy numbers were similar in both regions, significantly higher frequency of CNV and gain/loss were observed in genes located in the repeat-rich regions (Fig. 2A and fig. S7). Notably, presence/absence polymorphisms were 13 times as abundant in the gene-sparse compared to the gene-dense regions. In addition, even though SNP frequency was similar across the genomes, average dN/dS was significantly higher in gene-sparse regions, indicating more genes with signatures of positive selection (Fig. 2A). Indeed, 23% of the genes in the gene-sparse regions showed dN/dS > 1 in at least one of the resequenced genomes compared to only 11.5% of genes in the gene-dense regions. In total, 44.6% of the genes in the gene-sparse regions showed signatures of rapid evolution (deletion, duplication, or dN/dS > 1) compared to only 14.7% of the remaining genes. The uneven distribution in gene density in the *P. infestans* genome can be visualized with plots of two-dimensional bins of 5' and 3' flanking intergenic region (FIR) lengths (4). We adapted the plots to illustrate the relationships between gene density and polymorphism and confirmed the increased rates in the gene-sparse regions (Fig. 2B and fig. S8). We conclude that different regions of the examined genomes evolved at markedly different rates, with the gene-sparse, repeat-rich regions experiencing accelerated rates of evolution.

To gain insights into the functional basis of the uneven evolutionary rates detected in the gene-sparse versus gene-dense regions of the clade 1c species, we plotted genome-wide microarray expression data on the FIR length maps (fig. S9) (4). Gene-dense regions were enriched in genes induced in sporangia, the asexual spores that are produced by all *Phytophthora* species. In marked contrast, distribution patterns of genes induced during preinfection and infection stages indicate enrichment in genes located in gene-sparse loci (fig. S9). χ^2 tests revealed that the relationships between gene density (FIR length) and patterns of gene expression are significant (fig. S9 and table S3). We conclude that the gene-sparse, repeat-rich regions are highly enriched in genes induced in planta, therefore implicating host adaptation in genome evolution.

To assign biological functions to genes with accelerated rates of evolution that populate the gene-sparse, repeat-rich regions, we performed Markov clustering on the predicted proteome of *P. infestans* and implemented gene ontology mapping. Protein families (tribes) significantly enriched or deficient in genes that locate to gene-sparse regions or are rapidly evolving were identified with Fisher's exact test. In total, 811 tribes with five or more proteins were generated (44% of proteome) (figs. S10 and S11). Of these, 163 tribes were statistically enriched in genes from gene-sparse regions (Fig. 3A and fig. S12), 123 tribes

were enriched in fast-evolving genes (fig. S12), and 65 tribes were enriched for both criteria (Fig. 3B and fig. S12). As expected, several of these tribes (19 out of 65) consist of effector families (4, 11–13) (table S4). Other notable tribes include genes encoding various enzymes such as cell wall hydrolases and proteins related to epigenetic maintenance (Fig. 3B and table S4). Surprisingly, tribes annotated as histone and ribosomal RNA (rRNA) methyltransferases were particularly rich in genes located in gene-sparse regions and exhibiting presence/absence polymorphisms (table S4 and figs. S13 and S14). Several genes encoding DOT1-like and SET domain histone methyltransferases and SpoU-like rRNA methyltransferases are exceptional among genes involved in epigenetic maintenance for residing largely in gene-sparse regions and showing high rates of polymorphism (fig. S15).

Our study demonstrates that highly dynamic genome compartments enriched in noncoding sequences underpin accelerated gene evolution following host jumps. Gene-sparse regions that drive the extremely uneven architecture of the *P. infestans* genome are highly enriched in plant-induced genes, particularly effectors, therefore implicating host adaptation as a driving force of genome evolution in this lineage. In addition, we unexpectedly identified several genes involved in epigenetic processes, notably histone methyltransferases, as rapidly evolving residents of the gene-sparse regions. Histone methylation indirectly modulates gene expression in various eukaryotes (14, 15) and could underlie concerted and heritable gene induction patterns through long-range remodeling of chromatin structure (16). Histone acetylation and methylation are thought to be key regulators of gene expression in

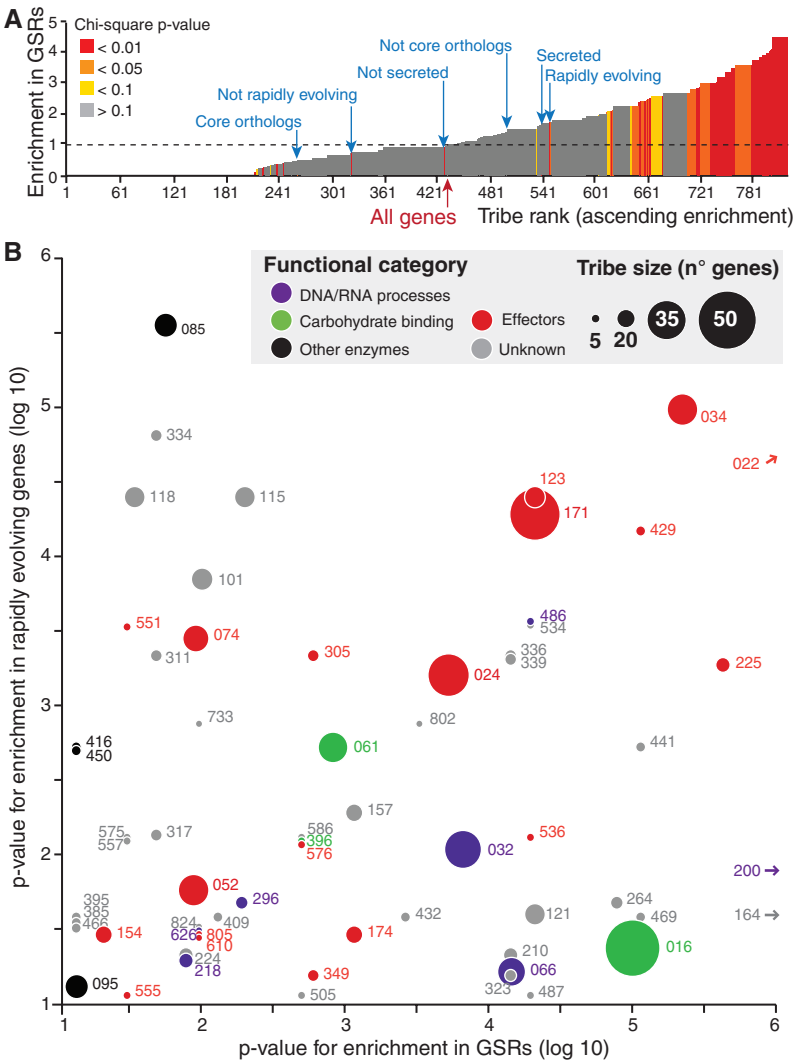


Fig. 3. Enrichment of *P. infestans* families (tribes) in genes residing in gene-sparse regions and rapidly evolving genes. (A) The 811 *P. infestans* tribes with five or more genes (x axis) ranked on the basis of ascending enrichment in GSR genes (y axis). P value of a χ^2 test for significance of enrichment is indicated. Additional gene categories (core/not core orthologs, secreted/not secreted, and rapidly/not rapidly evolving) are shown for reference. (B) P values of χ^2 tests for tribe enrichment in GSR genes (x axis) and rapidly evolving genes (y axis). Tribes with P values < 0.1 ($\log_{10}$) are shown. Bubble sizes are proportional to the number of genes in tribes. Bubble colors indicate functional categories. Numbers refer to tribe identifiers as listed in table S4.

P. infestans (17) and could modulate expression patterns of genes located in the gene-sparse regions. In addition, histone hypomethylation reduces DNA stability (18, 19) and may have contributed to genome plasticity in the *P. infestans* lineage by regulating transposon activity as well as genomic and expression variability (20, 21). Finally, understanding *P. infestans* genome evolution should prove useful in designing rational strategies for sustainable late blight disease management based on targeting the most evolutionarily stable genes in this lineage.

References and Notes

1. L. P. Kroon, F. T. Bakker, G. B. van den Bosch, P. J. Bonants, W. G. Flier, *Fungal Genet. Biol.* **41**, 766 (2004).
2. J. E. Blair, M. D. Coffey, S. Y. Park, D. M. Geiser, S. Kang, *Fungal Genet. Biol.* **45**, 266 (2008).
3. N. J. Grünwald, W. G. Flier, *Annu. Rev. Phytopathol.* **43**, 171 (2005).
4. B. J. Haas et al., *Nature* **461**, 393 (2009).
5. Materials and methods are available as supporting material on Science Online.
6. S. Yoon, Z. Xuan, V. Makarov, K. Ye, J. Sebat, *Genome Res.* **19**, 1586 (2009).
7. Z. Yang, R. Nielsen, *Mol. Biol. Evol.* **17**, 32 (2000).
8. Z. Liu et al., *Mol. Biol. Evol.* **22**, 659 (2005).
9. R. L. Allen et al., *Science* **306**, 1957 (2004).
10. J. Win et al., *Plant Cell* **19**, 2349 (2007).
11. S. Kamoun, *Annu. Rev. Phytopathol.* **44**, 41 (2006).
12. S. K. Oh et al., *Plant Cell* **21**, 2928 (2009).
13. M. Tian, E. Huitema, L. Da Cunha, T. Torto-Alalibo, S. Kamoun, *J. Biol. Chem.* **279**, 26370 (2004).
14. T. Kouzarides, *Curr. Opin. Genet. Dev.* **12**, 198 (2002).
15. Y. Zhang, D. Reinberg, *Genes Dev.* **15**, 2343 (2001).
16. L. I. Elizondo, P. Jafar-Nejad, J. M. Clewing, C. F. Boerkoel, *Curr. Genomics* **10**, 64 (2009).
17. P. van West et al., *Microbiology* **154**, 1482 (2008).
18. A. H. Peters et al., *Cell* **107**, 323 (2001).
19. J. C. Peng, G. H. Karpen, R. S. Hawley, *PLoS Genet.* **5**, e1000435 (2009).
20. D. W. Zeh, J. A. Zeh, Y. Ishida, *Bioessays* **31**, 715 (2009).
21. N. Elango, S. H. Kim, E. Vigoda, S. V. Yi, A. Sidow, *PLOS Comput. Biol.* **4**, e1000015 (2008).
22. We thank the Broad Institute Sequencing Platform and J. Pike for sequencing; G. Kessel and V. Vleeshouwers for biomaterial; and D. Weigel, J. Dangl, and J. Ecker for useful comments. This project was funded by the Gatsby Charitable Foundation, Marie-Curie IEF contract 255104, National Research Initiative of the U.S. Department of Agriculture grant 2006-35600-16623, NSF grant EF-0523670, and research funding program LOEWE of the Ministry of Research, Science and the Arts of Hesse (Germany). Sequences are deposited in GenBank under the submission accession numbers SRA02326–2329 and SRA024355 and in the Short Read Archive under study accession numbers ERP000341–344.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1540/DC1
Materials and Methods
Figs. S1 to S15
Tables S1 to S4
References

1 June 2010; accepted 21 October 2010
10.1126/science.1193070

Genome Expansion and Gene Loss in Powdery Mildew Fungi Reveal Tradeoffs in Extreme Parasitism

Pietro D. Spanu,^{1*} James C. Abbott,^{1†} Joelle Amselem,^{2,15†} Timothy A. Burgis,^{1†} Darren M. Soanes,^{3†} Kurt Stüber,^{4†} Emiel Ver Loren van Themaat,^{4†} James K. M. Brown,^{5‡} Sarah A. Butcher,^{1‡} Sarah J. Gurr,^{6‡} Marc-Henri Lebrun,^{15‡} Christopher J. Ridout,^{5‡} Paul Schulze-Lefert,^{4‡} Nicholas J. Talbot,^{3‡} Nahal Ahmadinejad,⁴ Christian Ametz,¹ Geraint R. Barton,¹ Mariam Benjdia,⁴ Przemyslaw Bidzinski,⁴ Laurence V. Bindschedler,⁷ Maike Both,¹ Marin T. Brewer,⁸ Lance Cadle-Davidson,^{9,10‡} Molly M. Cadle-Davidson,⁹ Jerome Collemare,^{2§} Rainer Cramer,⁷ Omer Frenkel,⁸ Dale Godfrey,¹¹ James Harriman,⁹ Claire Hoede,² Brian C. King,⁸ Sven Klages,¹² Jochen Kleemann,⁴ Daniela Knoll,⁴ Prasanna S. Koti,⁴ Jonathan Kreplak,² Francisco J. López-Ruiz,⁵ Xunli Lu,⁴ Takaki Maekawa,⁴ Sirapra Mahanil,⁹ Cristina Micali,¹ Michael G. Milgroom,⁸ Giovanni Montana,¹ Sandra Noir,^{4||} Richard J. O'Connell,⁴ Simone Oberhaensli,¹³ Francis Parlange,¹³ Carsten Pedersen,¹¹ Hadi Quesneville,² Richard Reinhardt,¹² Matthias Rott,⁴ Soledad Sacristán,¹⁴ Sarah M. Schmidt,^{4¶} Moritz Schön,⁴ Pari Skamnioti,⁶ Hans Sommer,⁴ Amber Stephens,⁴ Hiroyuki Takahara,⁴ Hans Thordal-Christensen,¹¹ Marielle Vigouroux,⁶ Ralf Weßling,⁴ Thomas Wicker,¹³ Ralph Panstruga^{4*}

Powdery mildews are phytopathogens whose growth and reproduction are entirely dependent on living plant cells. The molecular basis of this life-style, obligate biotrophy, remains unknown. We present the genome analysis of barley powdery mildew, *Blumeria graminis* f.sp. *hordei* (*Blumeria*), as well as a comparison with the analysis of two powdery mildews pathogenic on dicotyledonous plants. These genomes display massive retrotransposon proliferation, genome-size expansion, and gene losses. The missing genes encode enzymes of primary and secondary metabolism, carbohydrate-active enzymes, and transporters, probably reflecting their redundancy in an exclusively biotrophic life-style. Among the 248 candidate effectors of pathogenesis identified in the *Blumeria* genome, very few (less than 10) define a core set conserved in all three mildews, suggesting that most effectors represent species-specific adaptations.

Filamentous eukaryotes such as fungi and oomycetes (stramenopiles) are responsible for many serious plant diseases. Among these pathogens is a group of taxonomically diverse species, collectively termed obligate biotrophs, which only grow and reproduce on living plants. These microorganisms cause rusts, as well as downy and powdery mildews, and form dedicated invasive infection structures (haustoria) for nutrient uptake. Obligate biotrophs are found in two kingdoms (Stramenopila and Fungi) and in both major fungal phyla (Ascomycota and Basidiomycota), indicating that biotrophy is the result of convergent evolution.

The ascomycete powdery mildews infect ~10,000 angiosperm species, including many important crops (1). They form morphologically complex structures during asexual pathogenesis and produce fruiting bodies (cleistothecia), which develop after sexual reproduction (Fig. 1 and fig. S1).

We sequenced the haploid *Blumeria* genome with the use of Sanger protocols and second-generation methods (table S1) (2). We assembled the sequence reads with a combination of the cortex and CABOG (Celera assembler with the best overlap graph) (3) assemblers into 15,111 contigs (L_{50} : 18,024 bases; L_{50} is the length of

the smallest N_{50} contig, where N_{50} is the minimum number of contigs required to represent 50% of the genome) on 6898 supercontig scaffolds (L_{50} : 2,209,085 bases). The overall assembly size is 119,213,040 nucleotides (table S1). We estimate that the actual genome size is ~120 Mb, corresponding to 140-fold coverage of the *Blumeria* genome. We additionally generated draft genome assemblies (~eightfold coverage each) of two other powdery mildew species, *Erysiphe pisi* [pathogenic on pea (*Pisum sativum*)] and *Golovinomyces orontii* (pathogenic on *Arabidopsis thaliana*). Together with *Blumeria*, these species represent three of the five major tribes of the order Erysiphales, which diverged ~70 million years ago (4). We calculated that the genome sizes of the latter two species are ~151 and ~160 Mb, respectively (table

¹Department of Life Sciences, Imperial College London, London, UK. ²Institute National de la Recherche Agronomique (INRA), Unité de Recherche Genomique Info, Versailles, France. ³School of Biosciences, University of Exeter, Exeter, UK. ⁴Department of Plant Microbe Interactions, Max-Planck Institute for Plant Breeding Research, Cologne, Germany. ⁵Department of Disease and Stress Biology, John Innes Centre, Norwich, UK. ⁶Department of Plant Sciences, University of Oxford, Oxford, UK. ⁷Department of Chemistry, University of Reading, Reading, UK. ⁸Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY 14853, USA. ⁹U.S. Department of Agriculture–Agricultural Research Service Grape Genetics Research Unit, Geneva, NY 14853, USA. ¹⁰Department of Plant Pathology and Plant Microbe Biology, Cornell University, Geneva, NY 14456, USA. ¹¹Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark. ¹²Max-Planck Institute for Molecular Genetics, Berlin, Germany. ¹³Institute of Plant Biology, University of Zürich, Zürich, Switzerland. ¹⁴Centro de Biotecnología y Genómica de Plantas (UPM-INIA) and E.T.S.I. Agrónomos Universidad Politécnica de Madrid, Madrid, Spain. ¹⁵INRA, Unité BIOGER-CPP, Grignon, France.

*To whom correspondence should be addressed. E-mail: p.spanu@imperial.ac.uk (P.D.S.); panstrug@mpiz-koeln.mpg.de (R.P.)

†These authors contributed equally to this work.

‡These authors contributed equally to this work.

§Present address: Wageningen University, Laboratory of Phytopathology, Wageningen, Netherlands.

||Present address: Institut de Biologie Moléculaire des Plantes–CNRS, Strasbourg, France.

¶Present address: Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands.

P. infestans (17) and could modulate expression patterns of genes located in the gene-sparse regions. In addition, histone hypomethylation reduces DNA stability (18, 19) and may have contributed to genome plasticity in the *P. infestans* lineage by regulating transposon activity as well as genomic and expression variability (20, 21). Finally, understanding *P. infestans* genome evolution should prove useful in designing rational strategies for sustainable late blight disease management based on targeting the most evolutionarily stable genes in this lineage.

References and Notes

1. L. P. Kroon, F. T. Bakker, G. B. van den Bosch, P. J. Bonants, W. G. Flier, *Fungal Genet. Biol.* **41**, 766 (2004).
2. J. E. Blair, M. D. Coffey, S. Y. Park, D. M. Geiser, S. Kang, *Fungal Genet. Biol.* **45**, 266 (2008).
3. N. J. Grünwald, W. G. Flier, *Annu. Rev. Phytopathol.* **43**, 171 (2005).
4. B. J. Haas et al., *Nature* **461**, 393 (2009).
5. Materials and methods are available as supporting material on Science Online.
6. S. Yoon, Z. Xuan, V. Makarov, K. Ye, J. Sebat, *Genome Res.* **19**, 1586 (2009).
7. Z. Yang, R. Nielsen, *Mol. Biol. Evol.* **17**, 32 (2000).
8. Z. Liu et al., *Mol. Biol. Evol.* **22**, 659 (2005).
9. R. L. Allen et al., *Science* **306**, 1957 (2004).
10. J. Win et al., *Plant Cell* **19**, 2349 (2007).
11. S. Kamoun, *Annu. Rev. Phytopathol.* **44**, 41 (2006).
12. S. K. Oh et al., *Plant Cell* **21**, 2928 (2009).
13. M. Tian, E. Huitema, L. Da Cunha, T. Torto-Alalibo, S. Kamoun, *J. Biol. Chem.* **279**, 26370 (2004).
14. T. Kouzarides, *Curr. Opin. Genet. Dev.* **12**, 198 (2002).
15. Y. Zhang, D. Reinberg, *Genes Dev.* **15**, 2343 (2001).
16. L. I. Elizondo, P. Jafar-Nejad, J. M. Clewing, C. F. Boerkoel, *Curr. Genomics* **10**, 64 (2009).
17. P. van West et al., *Microbiology* **154**, 1482 (2008).
18. A. H. Peters et al., *Cell* **107**, 323 (2001).
19. J. C. Peng, G. H. Karpen, R. S. Hawley, *PLoS Genet.* **5**, e1000435 (2009).
20. D. W. Zeh, J. A. Zeh, Y. Ishida, *Bioessays* **31**, 715 (2009).
21. N. Elango, S. H. Kim, E. Vigoda, S. V. Yi, A. Sidow, *PLOS Comput. Biol.* **4**, e1000015 (2008).
22. We thank the Broad Institute Sequencing Platform and J. Pike for sequencing; G. Kessel and V. Vleeshouwers for biomaterial; and D. Weigel, J. Dangl, and J. Ecker for useful comments. This project was funded by the Gatsby Charitable Foundation, Marie-Curie IEF contract 255104, National Research Initiative of the U.S. Department of Agriculture grant 2006-35600-16623, NSF grant EF-0523670, and research funding program LOEWE of the Ministry of Research, Science and the Arts of Hesse (Germany). Sequences are deposited in GenBank under the submission accession numbers SRA02326–2329 and SRA024355 and in the Short Read Archive under study accession numbers ERP000341–344.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1540/DC1
Materials and Methods
Figs. S1 to S15
Tables S1 to S4
References

1 June 2010; accepted 21 October 2010
10.1126/science.1193070

Genome Expansion and Gene Loss in Powdery Mildew Fungi Reveal Tradeoffs in Extreme Parasitism

Pietro D. Spanu,^{1*} James C. Abbott,^{1†} Joelle Amselem,^{2,15†} Timothy A. Burgis,^{1†} Darren M. Soanes,^{3†} Kurt Stüber,^{4†} Emiel Ver Loren van Themaat,^{4†} James K. M. Brown,^{5‡} Sarah A. Butcher,^{1‡} Sarah J. Gurr,^{6‡} Marc-Henri Lebrun,^{15‡} Christopher J. Ridout,^{5‡} Paul Schulze-Lefert,^{4‡} Nicholas J. Talbot,^{3‡} Nahal Ahmadinejad,⁴ Christian Ametz,¹ Geraint R. Barton,¹ Mariam Benjdia,⁴ Przemyslaw Bidzinski,⁴ Laurence V. Bindschedler,⁷ Maike Both,¹ Marin T. Brewer,⁸ Lance Cadle-Davidson,^{9,10‡} Molly M. Cadle-Davidson,⁹ Jerome Collemare,^{2§} Rainer Cramer,⁷ Omer Frenkel,⁸ Dale Godfrey,¹¹ James Harriman,⁹ Claire Hoede,² Brian C. King,⁸ Sven Klages,¹² Jochen Kleemann,⁴ Daniela Knoll,⁴ Prasanna S. Koti,⁴ Jonathan Kreplak,² Francisco J. López-Ruiz,⁵ Xunli Lu,⁴ Takaki Maekawa,⁴ Sirapra Mahanil,⁹ Cristina Micali,¹ Michael G. Milgroom,⁸ Giovanni Montana,¹ Sandra Noir,^{4||} Richard J. O'Connell,⁴ Simone Oberhaensli,¹³ Francis Parlange,¹³ Carsten Pedersen,¹¹ Hadi Quesneville,² Richard Reinhardt,¹² Matthias Rott,⁴ Soledad Sacristán,¹⁴ Sarah M. Schmidt,^{4¶} Moritz Schön,⁴ Pari Skamnioti,⁶ Hans Sommer,⁴ Amber Stephens,⁴ Hiroyuki Takahara,⁴ Hans Thordal-Christensen,¹¹ Marielle Vigouroux,⁶ Ralf Weßling,⁴ Thomas Wicker,¹³ Ralph Panstruga^{4*}

Powdery mildews are phytopathogens whose growth and reproduction are entirely dependent on living plant cells. The molecular basis of this life-style, obligate biotrophy, remains unknown. We present the genome analysis of barley powdery mildew, *Blumeria graminis* f.sp. *hordei* (*Blumeria*), as well as a comparison with the analysis of two powdery mildews pathogenic on dicotyledonous plants. These genomes display massive retrotransposon proliferation, genome-size expansion, and gene losses. The missing genes encode enzymes of primary and secondary metabolism, carbohydrate-active enzymes, and transporters, probably reflecting their redundancy in an exclusively biotrophic life-style. Among the 248 candidate effectors of pathogenesis identified in the *Blumeria* genome, very few (less than 10) define a core set conserved in all three mildews, suggesting that most effectors represent species-specific adaptations.

Filamentous eukaryotes such as fungi and oomycetes (stramenopiles) are responsible for many serious plant diseases. Among these pathogens is a group of taxonomically diverse species, collectively termed obligate biotrophs, which only grow and reproduce on living plants. These microorganisms cause rusts, as well as downy and powdery mildews, and form dedicated invasive infection structures (haustoria) for nutrient uptake. Obligate biotrophs are found in two kingdoms (Stramenopila and Fungi) and in both major fungal phyla (Ascomycota and Basidiomycota), indicating that biotrophy is the result of convergent evolution.

The ascomycete powdery mildews infect ~10,000 angiosperm species, including many important crops (1). They form morphologically complex structures during asexual pathogenesis and produce fruiting bodies (cleistothecia), which develop after sexual reproduction (Fig. 1 and fig. S1).

We sequenced the haploid *Blumeria* genome with the use of Sanger protocols and second-generation methods (table S1) (2). We assembled the sequence reads with a combination of the cortex and CABOG (Celera assembler with the best overlap graph) (3) assemblers into 15,111 contigs (L_{50} : 18,024 bases; L_{50} is the length of

the smallest N_{50} contig, where N_{50} is the minimum number of contigs required to represent 50% of the genome) on 6898 supercontig scaffolds (L_{50} : 2,209,085 bases). The overall assembly size is 119,213,040 nucleotides (table S1). We estimate that the actual genome size is ~120 Mb, corresponding to 140-fold coverage of the *Blumeria* genome. We additionally generated draft genome assemblies (~eightfold coverage each) of two other powdery mildew species, *Erysiphe pisi* [pathogenic on pea (*Pisum sativum*)] and *Golovinomyces orontii* (pathogenic on *Arabidopsis thaliana*). Together with *Blumeria*, these species represent three of the five major tribes of the order Erysiphales, which diverged ~70 million years ago (4). We calculated that the genome sizes of the latter two species are ~151 and ~160 Mb, respectively (table

¹Department of Life Sciences, Imperial College London, London, UK. ²Institute National de la Recherche Agronomique (INRA), Unité de Recherche Genomique Info, Versailles, France. ³School of Biosciences, University of Exeter, Exeter, UK. ⁴Department of Plant Microbe Interactions, Max-Planck Institute for Plant Breeding Research, Cologne, Germany. ⁵Department of Disease and Stress Biology, John Innes Centre, Norwich, UK. ⁶Department of Plant Sciences, University of Oxford, Oxford, UK. ⁷Department of Chemistry, University of Reading, Reading, UK. ⁸Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY 14853, USA. ⁹U.S. Department of Agriculture–Agricultural Research Service Grape Genetics Research Unit, Geneva, NY 14853, USA. ¹⁰Department of Plant Pathology and Plant Microbe Biology, Cornell University, Geneva, NY 14456, USA. ¹¹Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark. ¹²Max-Planck Institute for Molecular Genetics, Berlin, Germany. ¹³Institute of Plant Biology, University of Zürich, Zürich, Switzerland. ¹⁴Centro de Biotecnología y Genómica de Plantas (UPM-INIA) and E.T.S.I. Agrónomos Universidad Politécnica de Madrid, Madrid, Spain. ¹⁵INRA, Unité BIOGER-CPP, Grignon, France.

*To whom correspondence should be addressed. E-mail: p.spanu@imperial.ac.uk (P.D.S.); panstrug@mpiz-koeln.mpg.de (R.P.)

†These authors contributed equally to this work.

‡These authors contributed equally to this work.

§Present address: Wageningen University, Laboratory of Phytopathology, Wageningen, Netherlands.

||Present address: Institut de Biologie Moléculaire des Plantes–CNRS, Strasbourg, France.

¶Present address: Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands.

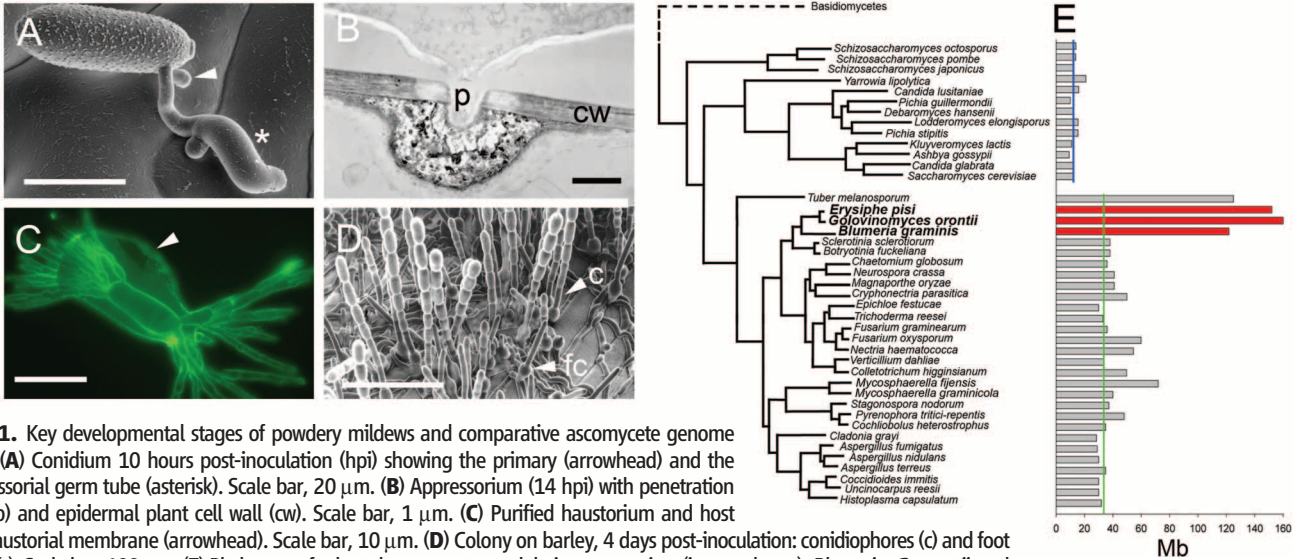


Fig. 1. Key developmental stages of powdery mildews and comparative ascomycete genome sizes. **(A)** Conidium 10 hours post-inoculation (hpi) showing the primary (arrowhead) and the appressorial germ tube (asterisk). Scale bar, 20 μ m. **(B)** Appressorium (14 hpi) with penetration peg (p) and epidermal plant cell wall (cw). Scale bar, 1 μ m. **(C)** Purified haustorium and host periaustorial membrane (arrowhead). Scale bar, 10 μ m. **(D)** Colony on barley, 4 days post-inoculation: conidiophores (c) and foot cells (fc). Scale bar, 100 μ m. **(E)** Phylogeny of selected ascomycetes and their genome sizes (in megabases). *Blumeria*, *G. orontii*, and *E. pisi* are shown in red. The median genome sizes of the hemiascomycetes (blue vertical line, 12.3 Mb) and euascomycetes (green vertical line, 36.7 Mb) are also shown.

S1). Thus, the genome size of each of the mildews is more than four times larger than the median of other ascomycetes (Fig. 1E). We first annotated the *Blumeria* genome using ab initio gene finders followed by extensive manual curation (table S2). The actual number of curated genes is 5854, which is at the lower end of the range of fungal genomes.

The comparatively low gene number and the inability of the parasite to grow in vitro suggest that the mildew genomes may lack genes typically present in autotrophic ascomycetes. We systematically searched for genes absent in the mildews but present in baker's yeast (*Saccharomyces cerevisiae*) and the phytopathogens *Colletotrichum higginsianum*, *Magnaporthe oryzae*, and *Sclerotinia sclerotiorum* (Fig. 2A). We identified 90 yeast genes by this procedure and 9 additional common ascomycete genes by manual inspection that are missing in the genome assemblies of all three mildews [hereafter referred to as missing ascomycete core genes (MACGs)]. It is unlikely that these gene sets were missed as a result of incomplete genome coverage because (i) the 140 $\times$ *Blumeria* assembly encompasses >99% of the conserved gene space (table S1), and (ii) these genes are missing in all three assemblies. The MACGs represent a diverse set of metabolic and regulatory proteins, affecting multiple processes and pathways (for example, thiamine biosynthesis), and a considerable subset of MACGs (57 to 77%) also seems to be absent in other obligate biotrophic phytopathogens (fig. S2 and table S3).

The existence of MACGs raises the possibility that their expression may be detrimental to biotrophy. To test this, we determined expression of MACG homologs in *C. higginsianum*, a phytopathogenic fungus that first employs a biotrophic growth mode and later switches to necrotrophic pathogenesis, involving host cell killing. Analysis of the *C. higginsianum* transcriptome revealed that most of the MACG homologs that we tested (26 out of 32) are expressed during the biotrophic

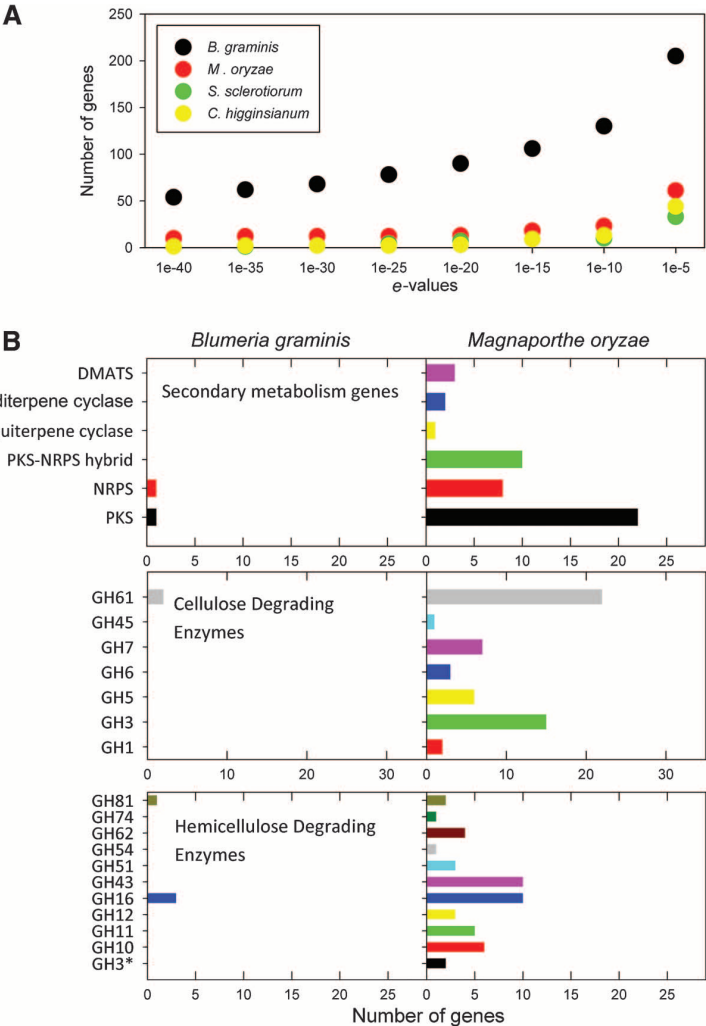


Fig. 2. Gene losses in powdery mildews. **(A)** Number of missing *S. cerevisiae* proteins in one fungus compared with the three others as a function of TBLASTN e-values. **(B)** Number of genes devoted to secondary metabolism and genes encoding cellulose- or hemicellulose-degrading enzymes in *Blumeria* (left) and *M. oryzae* (right). DMATS, dimethylallyl diphosphate tryptophan synthase; GH1, glycosyl hydrolase.

stage (fig. S3). Their expression is, therefore, unlikely to be detrimental to biotrophic growth.

Although the vast majority of genes encoding enzymes of primary metabolism are retained, notable exceptions include anaerobic fermentation, biosynthesis of glycerol from glycolytic intermediates, and inorganic nitrogen (nitrate) assimilation. These deficiencies are consistent with an exclusively aerobic, parasitic life-style on aerial plant organs, the production of solutes for the generation of osmotic pressure during plant cell wall penetration from triacyl glycerol breakdown, and the assimilation of organic nitrogen in the form of host-derived amino acids.

Filamentous fungi generally produce an array of secondary metabolites, some of which are involved in pathogenesis (5). Key enzymes that catalyze their biosynthesis include polyketide synthases (PKSs), modular nonribosomal peptide synthetases (NRPSs), terpene cyclases, and dimethylallyl diphosphate tryptophan synthases (6). *Blumeria* encodes only two such proteins (one PKS and one NRPS), the lowest number known in fungi (Fig. 2B and fig. S4). We hypothesize that *Blumeria* synthesizes only one iron siderophore and one simple polyketide, possibly the pigment observed on cleistothecia (fig. S1, G and H). Similar trends are observed in other biotrophs, such as the basidiomycete *Ustilago maydis* and the plant symbiotic fungus *Tuber melanosporum*. Therefore, it appears that biotrophy is associated with a convergent loss of secondary metabolic enzymes. We also noted a marked reduction in genes encoding specific subfamilies of transporters (fig. S5), which typically function in secretion of toxins

into the host and extrusion of host defense compounds in necrotrophic fungi (7).

Unlike other known plant pathogenic fungi, *Blumeria* has an extremely reduced set of carbohydrate active enzymes devoted to plant cell wall depolymerization (Fig. 2B and fig. S6) (8). We found no canonical cellulose-, xylan-, or pectin-degrading enzymes. Other biotrophic phytopathogens, such as *U. maydis* and *Puccinia graminis*, also possess reduced enzyme systems for degradation of the plant cell wall, but both species have predicted cellulases and xylanases. An example of structural proteins lacking in the mildews are the hydrophobins, a class of cell wall proteins that are typically present in fungi (9).

We found a massive proliferation of transposable elements (TEs) (table S4). In *Blumeria*, where TEs account for 64% of the genome size, the most abundant families comprise non-long terminal repeat (LTR) retrotransposons lacking LTRs (fig. S7). TEs were evenly distributed throughout the *Blumeria* genome, with no evidence of clustering of particular TEs (fig. S8). Protein-coding genes are typically in small clusters (2 to 10 genes) interspersed between extended stretches of TEs. In all three powdery mildew genomes, genes required for repeat-induced point mutations (RIPs) are absent, whereas all components known to be necessary for mitotic and meiotic silencing are present (table S5). Thus, dysfunctionality of the RIP pathway has probably contributed to genome-size inflation, and extensive retrotransposition (rather than gradual pseudogenization) may account for the observed gene losses and reshuffling. An ex-

ample of the latter is the otherwise-conserved mating type (*MAT*) locus (10). In all other ascomycetes, *MAT* genes are flanked by a conserved cluster of functionally unrelated genes. Although the micro-synteny of these genes is retained in the *Blumeria* genome (fig. S9), *MAT* was found on a different supercontig, indicating physical separation.

In addition to >1350 paralog copies of the previously described atypical avirulence genes *AVRk1* and *AVRa10* (11), we predicted 248 *Blumeria* proteins with a signal peptide (SP) but lacking any transmembrane domain and BLAST (Basic Local Alignment Search Tool) hit outside the mildews, thus representing candidates for secreted effector proteins (CSEPs) (12). The CSEPs have distinctive features (table S6) and show great sequence diversity with few members grouping in small families (Fig. 3A). We noted no obvious clustering of CSEPs within the *Blumeria* contigs. Approximately 80% harbor a recently identified N-terminal tripeptide motif, termed “YxC,” (13), that typically occurs within the first 30 amino acids after the predicted SP cleavage site. Searches in the *E. pisi* and *G. orontii* genomes revealed that the vast majority of CSEPs are confined to *Blumeria* (Fig. 3A and table S6). Thus, powdery mildew genomes preferentially harbor species- and/or tribe-specific innovations, which possibly evolved in the context of cospeciation with their plant hosts (11). Upon comparison of global gene expression in haustoria (Fig. 1C) and epiphytic structures (Fig. 1D), we observed preferential expression of the majority of the CSEPs (79%) in haustoria (Fig. 3B), suggesting they have specific functions in biotrophic pathogenesis (14).

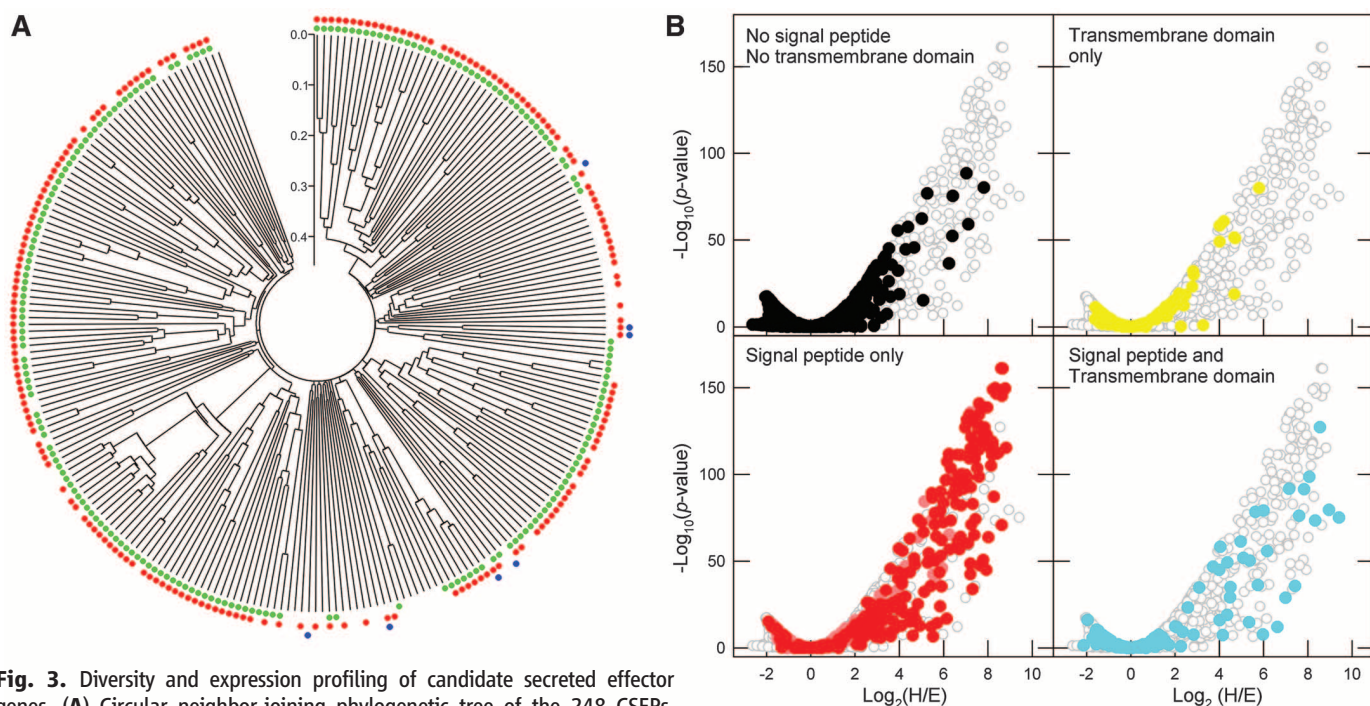


Fig. 3. Diversity and expression profiling of candidate secreted effector genes. **(A)** Circular neighbor-joining phylogenetic tree of the 248 CSEPs. Scale, amino acid substitutions per site; green, CSEPs harboring the YxC motif; blue, CSEPs conserved in *E. pisi* and/or *G. orontii*; red, CSEPs predominantly expressed in haustoria. **(B)** Global gene expression in haustoria (H) versus epiphytic structures (E). Relative abundance of each gene plotted versus the

p-value as a measure of the statistical significance. White circles, all gene models; black, no SP and no TM domain; yellow, TM only; red, CSEPs; pink, genes with BLASTP hits in the National Center for Biotechnology Information nr database and SP only; light blue, both SP and TM domains.

We detected common genomic hallmarks in the powdery mildews associated with obligate biotrophy. These include gene losses and extensive gene reshuffling correlated with expansion in (retro-)transposon number and genome size. Together, these hallmarks may represent a tradeoff between advantages of increased genetic variation independent of sexual recombination and irreversible deletion of genes dispensable for biotrophy. Hence, their evolution provides a notable example of Dollo's law of evolutionary irreversibility (15). This may explain why powdery mildews and possibly other biotrophic parasites became obligate.

References and Notes

1. D. A. Glawe, *Annu. Rev. Phytopathol.* **46**, 27 (2008).
2. Materials and methods are available as supporting material on Science Online.
3. J. R. Miller *et al.*, *Bioinformatics* **24**, 2818 (2008).
4. S. Takamatsu, *Mycoscience* **45**, 147 (2004).
5. N. Möbius, C. Hertweck, *Curr. Opin. Plant Biol.* **12**, 390 (2009).
6. J. Collemare, A. Billard, H. U. Böhnert, M. H. Lebrun, *Mycol. Res.* **112**, 207 (2008).
7. J. Glazebrook, *Annu. Rev. Phytopathol.* **43**, 205 (2005).
8. B. L. Cantarel *et al.*, *Nucleic Acids Res.* **37** (database issue), D233 (2009).
9. H. A. B. Wösten, *Annu. Rev. Microbiol.* **55**, 625 (2001).
10. R. Debuchy, B. Turgeon, in *The Mycota I: Growth, Differentiation and Sexuality*, U. Kües, R. Fischer, Eds. (Springer, Berlin, 2006), vol. 1, pp. 293–323.
11. S. Sacristán *et al.*, *PLoS ONE* **4**, e7463 (2009).
12. R. Panstruga, P. N. Dodds, *Science* **324**, 748 (2009).
13. D. Godfrey *et al.*, *BMC Genomics* **11**, 317 (2010).
14. A.-M. Catanzariti, P. N. Dodds, G. J. Lawrence, M. A. Ayliffe, J. G. Ellis, *Plant Cell* **18**, 243 (2006).
15. C. R. Marshall, E. C. Raff, R. A. Raff, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12283 (1994).
16. We thank B. Thomas, T. Carver, and D. Grunewald for providing scanning electron micrographs. This work was supported by funds from the Biotechnology and Biological Sciences Research Council [grant BB/E000983/1/1], INRA, the BioExploit European Union Framework 6 project, Deutsche Forschungsgemeinschaft (priority program SPP1212), the Leverhulme Trust, and the Max Planck Society. GenBank project identification numbers are as follows: *Blumeria*, 28821; *E. pisi*, 50315; and *G. orontii*, 50317.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1543/DC1
Materials and Methods
SOM Text
Figs. S1 to S9
Tables S1 to S6
References

2 July 2010; accepted 1 November 2010
10.1126/science.1194573

Pathogenicity Determinants in Smut Fungi Revealed by Genome Comparison

Jan Schirawski,^{1,2*} Gertrud Mannhaupt,^{1,3} Karin Münch,¹ Thomas Brefort,^{1†} Kerstin Schipper,¹ Gunther Doehlemann,¹ Maurizio Di Stasio,¹ Nicole Rössel,¹ Artemio Mendoza-Mendoza,^{1‡} Doris Pester,^{1§} Olaf Müller,^{1||} Britta Winterberg,^{1¶} Elmar Meyer,¹ Hassan Ghareeb,^{1*} Theresa Wollenberg,^{1*} Martin Münsterkötter,³ Philip Wong,³ Mathias Walter,³ Eva Stukenbrock,¹ Ulrich Güldener,³ Regine Kahmann^{1#}

Biotrophic pathogens, such as the related maize pathogenic fungi *Ustilago maydis* and *Sporisorium reilianum*, establish an intimate relationship with their hosts by secreting protein effectors. Because secreted effectors interacting with plant proteins should rapidly evolve, we identified variable genomic regions by sequencing the genome of *S. reilianum* and comparing it with the *U. maydis* genome. We detected 43 regions of low sequence conservation in otherwise well-conserved syntenic genomes. These regions primarily encode secreted effectors and include previously identified virulence clusters. By deletion analysis in *U. maydis*, we demonstrate a role in virulence for four previously unknown diversity regions. This highlights the power of comparative genomics of closely related species for identification of virulence determinants.

Smut fungi are biotrophic pathogens causing disease in a number of agriculturally important crop plants. *Ustilago maydis* and the related fungus *Sporisorium reilianum* both parasitize maize (1, 2). Their life cycle leading to the infectious form is similar (2, 3); however, shortly after infection *U. maydis* locally induces tumors on all aerial parts of the plant, whereas *S. reilianum* spreads systemically and causes symptoms in male and female inflorescences only (Fig. 1). Both *S. reilianum* and *U. maydis* establish an intimate communication with their host through secreted protein effectors that enable biotrophic development (3, 4). Effector proteins like *U. maydis* Pep1 can suppress plant defense responses (5). Additional effector genes were identified in the genome as genes encoding *U. maydis*-specific secreted proteins, most of which are up-regulated during host colonization (3). Many of these effector genes are clustered, and deletion of five of these clusters affected virulence in seedlings (3). Some cluster genes are induced in specific plant organs, and respective cluster mutants show altered virulence depending on the host tissue infected (6). In plant parasitic oomycetes, genes for effector proteins are under diversifying selection and occur in highly flexible genomic regions (7). In accordance with

this emerging picture of plant-pathogen communication via rapidly evolving effector proteins, we hypothesized that virulence-associated *U. maydis* genes might be identified as genomic regions with high sequence variability in closely related smut species.

To identify regions of high diversity in the *U. maydis* genome, we sequenced the genome of *S. reilianum* strain SRZ2 (8). The *S. reilianum* genome assembly covers 97% of the 18.7-Mb genome (9). As in *U. maydis* (3), the genome is organized in 23 chromosomes, to which 6648 gene models could be assigned after manual annotation. The genomes of *U. maydis* and *S. reilianum* exhibit a remarkable degree of synteny (Fig. 2A) (10) despite an average amino acid identity of predicted proteins of only 74.2% (Fig. 2B). Interestingly, some chromosome ends are extended by up to 20 genes in *U. maydis* compared with ends in *S. reilianum*. About 90% of these chromosome end-associated genes do not carry any functional annotation and no enrichment for secreted effectors is evident (fig. S1), whereas the others likely encode enzymes for secondary metabolism (table S1). Because orthologs of these genes are lacking in *S. reilianum*, their presence is likely dispensable for virulence. Compared with an average amino acid identity of 76% for nonsecreted proteins,

secreted proteins in both organisms display an average identity of only 62% and are enriched among the weakly conserved proteins (Fig. 2B). This suggests that genes coding for secreted proteins are subject to more rapid evolution.

Manual sequence comparisons of predicted gene models of *S. reilianum* and *U. maydis* led to a reannotation of more than 300 gene models of *U. maydis* (table S2). The *S. reilianum* genome has a 5.7% higher GC content than the *U. maydis* genome and a 5% higher coding potential (table S2). More than 99% of all InterPro (www.ebi.ac.uk/interpro/) domains are equally or close to equally represented in the two genomes, suggesting that the biosynthetic repertoire of both species is comparable. However, *S. reilianum* contains three putative RNA-dependent RNA polymerase genes (table S3). A search for other components (11, 12) of a putative RNA interference (RNAi) machinery in *S. reilianum* identified homologs of *dicer* and *argonaute* (table S3). These genes all lie in highly syntenic regions (10); however, the corresponding intergenic regions in *U. maydis* lack traces of the

¹Department of Organismic Interactions, Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch Straße 10, 35043 Marburg, Germany. ²Department for Molecular Biology of Plant-Microbe Interactions, Albrecht-von-Haller Institute for Plant Sciences, Georg-August-Universität Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany. ³Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany.

*Present address: Department for Molecular Biology of Plant-Microbe Interactions, Albrecht-von-Haller Institute for Plant Sciences, Georg-August-Universität Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany.

†Present address: Febit Biomed GmbH, Im Neuenheimer Feld 519, 69120 Heidelberg, Germany.

‡Present address: Bio-Protection Research Centre, Post Office Box 84, Lincoln University, Lincoln 7647, New Zealand.

§Present address: Institute for Plant Health, Austrian Agency for Health and Food Safety (AGES), Spargelfeldstraße 191, 1226 Wien, Austria.

||Present address: Roy J. Carver Center for Comparative Genomics, University of Iowa, Department of Biological Sciences, 303 Biology Building, Iowa City, IA 52242, USA.

¶Present address: Research School of Biology, Building 41, Linnaeus Way, Australian National University, Canberra, ACT 0200, Australia.

#To whom correspondence should be addressed. E-mail: Kahmann@mpi-marburg.mpg.de

We detected common genomic hallmarks in the powdery mildews associated with obligate biotrophy. These include gene losses and extensive gene reshuffling correlated with expansion in (retro-)transposon number and genome size. Together, these hallmarks may represent a tradeoff between advantages of increased genetic variation independent of sexual recombination and irreversible deletion of genes dispensable for biotrophy. Hence, their evolution provides a notable example of Dollo's law of evolutionary irreversibility (15). This may explain why powdery mildews and possibly other biotrophic parasites became obligate.

References and Notes

1. D. A. Glawe, *Annu. Rev. Phytopathol.* **46**, 27 (2008).
2. Materials and methods are available as supporting material on Science Online.
3. J. R. Miller *et al.*, *Bioinformatics* **24**, 2818 (2008).
4. S. Takamatsu, *Mycoscience* **45**, 147 (2004).
5. N. Möbius, C. Hertweck, *Curr. Opin. Plant Biol.* **12**, 390 (2009).
6. J. Collemare, A. Billard, H. U. Böhnert, M. H. Lebrun, *Mycol. Res.* **112**, 207 (2008).
7. J. Glazebrook, *Annu. Rev. Phytopathol.* **43**, 205 (2005).
8. B. L. Cantarel *et al.*, *Nucleic Acids Res.* **37** (database issue), D233 (2009).
9. H. A. B. Wösten, *Annu. Rev. Microbiol.* **55**, 625 (2001).
10. R. Debuchy, B. Turgeon, in *The Mycota I: Growth, Differentiation and Sexuality*, U. Kües, R. Fischer, Eds. (Springer, Berlin, 2006), vol. 1, pp. 293–323.
11. S. Sacristán *et al.*, *PLoS ONE* **4**, e7463 (2009).
12. R. Panstruga, P. N. Dodds, *Science* **324**, 748 (2009).
13. D. Godfrey *et al.*, *BMC Genomics* **11**, 317 (2010).
14. A.-M. Catanzariti, P. N. Dodds, G. J. Lawrence, M. A. Ayliffe, J. G. Ellis, *Plant Cell* **18**, 243 (2006).
15. C. R. Marshall, E. C. Raff, R. A. Raff, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12283 (1994).
16. We thank B. Thomas, T. Carver, and D. Grunewald for providing scanning electron micrographs. This work was supported by funds from the Biotechnology and Biological Sciences Research Council [grant BB/E000983/1/1], INRA, the BioExploit European Union Framework 6 project, Deutsche Forschungsgemeinschaft (priority program SPP1212), the Leverhulme Trust, and the Max Planck Society. GenBank project identification numbers are as follows: *Blumeria*, 28821; *E. pisi*, 50315; and *G. orontii*, 50317.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1543/DC1
Materials and Methods
SOM Text
Figs. S1 to S9
Tables S1 to S6
References

2 July 2010; accepted 1 November 2010
10.1126/science.1194573

Pathogenicity Determinants in Smut Fungi Revealed by Genome Comparison

Jan Schirawski,^{1,2*} Gertrud Mannhaupt,^{1,3} Karin Münch,¹ Thomas Brefort,^{1†} Kerstin Schipper,¹ Gunther Doehlemann,¹ Maurizio Di Stasio,¹ Nicole Rössel,¹ Artemio Mendoza-Mendoza,^{1‡} Doris Pester,^{1§} Olaf Müller,^{1||} Britta Winterberg,^{1¶} Elmar Meyer,¹ Hassan Ghareeb,^{1*} Theresa Wollenberg,^{1*} Martin Münsterkötter,³ Philip Wong,³ Mathias Walter,³ Eva Stukenbrock,¹ Ulrich Güldener,³ Regine Kahmann^{1#}

Biotrophic pathogens, such as the related maize pathogenic fungi *Ustilago maydis* and *Sporisorium reilianum*, establish an intimate relationship with their hosts by secreting protein effectors. Because secreted effectors interacting with plant proteins should rapidly evolve, we identified variable genomic regions by sequencing the genome of *S. reilianum* and comparing it with the *U. maydis* genome. We detected 43 regions of low sequence conservation in otherwise well-conserved syntenic genomes. These regions primarily encode secreted effectors and include previously identified virulence clusters. By deletion analysis in *U. maydis*, we demonstrate a role in virulence for four previously unknown diversity regions. This highlights the power of comparative genomics of closely related species for identification of virulence determinants.

Smut fungi are biotrophic pathogens causing disease in a number of agriculturally important crop plants. *Ustilago maydis* and the related fungus *Sporisorium reilianum* both parasitize maize (1, 2). Their life cycle leading to the infectious form is similar (2, 3); however, shortly after infection *U. maydis* locally induces tumors on all aerial parts of the plant, whereas *S. reilianum* spreads systemically and causes symptoms in male and female inflorescences only (Fig. 1). Both *S. reilianum* and *U. maydis* establish an intimate communication with their host through secreted protein effectors that enable biotrophic development (3, 4). Effector proteins like *U. maydis* Pep1 can suppress plant defense responses (5). Additional effector genes were identified in the genome as genes encoding *U. maydis*-specific secreted proteins, most of which are up-regulated during host colonization (3). Many of these effector genes are clustered, and deletion of five of these clusters affected virulence in seedlings (3). Some cluster genes are induced in specific plant organs, and respective cluster mutants show altered virulence depending on the host tissue infected (6). In plant parasitic oomycetes, genes for effector proteins are under diversifying selection and occur in highly flexible genomic regions (7). In accordance with

this emerging picture of plant-pathogen communication via rapidly evolving effector proteins, we hypothesized that virulence-associated *U. maydis* genes might be identified as genomic regions with high sequence variability in closely related smut species.

To identify regions of high diversity in the *U. maydis* genome, we sequenced the genome of *S. reilianum* strain SRZ2 (8). The *S. reilianum* genome assembly covers 97% of the 18.7-Mb genome (9). As in *U. maydis* (3), the genome is organized in 23 chromosomes, to which 6648 gene models could be assigned after manual annotation. The genomes of *U. maydis* and *S. reilianum* exhibit a remarkable degree of synteny (Fig. 2A) (10) despite an average amino acid identity of predicted proteins of only 74.2% (Fig. 2B). Interestingly, some chromosome ends are extended by up to 20 genes in *U. maydis* compared with ends in *S. reilianum*. About 90% of these chromosome end-associated genes do not carry any functional annotation and no enrichment for secreted effectors is evident (fig. S1), whereas the others likely encode enzymes for secondary metabolism (table S1). Because orthologs of these genes are lacking in *S. reilianum*, their presence is likely dispensable for virulence. Compared with an average amino acid identity of 76% for nonsecreted proteins,

secreted proteins in both organisms display an average identity of only 62% and are enriched among the weakly conserved proteins (Fig. 2B). This suggests that genes coding for secreted proteins are subject to more rapid evolution.

Manual sequence comparisons of predicted gene models of *S. reilianum* and *U. maydis* led to a reannotation of more than 300 gene models of *U. maydis* (table S2). The *S. reilianum* genome has a 5.7% higher GC content than the *U. maydis* genome and a 5% higher coding potential (table S2). More than 99% of all InterPro (www.ebi.ac.uk/interpro/) domains are equally or close to equally represented in the two genomes, suggesting that the biosynthetic repertoire of both species is comparable. However, *S. reilianum* contains three putative RNA-dependent RNA polymerase genes (table S3). A search for other components (11, 12) of a putative RNA interference (RNAi) machinery in *S. reilianum* identified homologs of *dicer* and *argonaute* (table S3). These genes all lie in highly syntenic regions (10); however, the corresponding intergenic regions in *U. maydis* lack traces of the

¹Department of Organismic Interactions, Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch Straße 10, 35043 Marburg, Germany. ²Department for Molecular Biology of Plant-Microbe Interactions, Albrecht-von-Haller Institute for Plant Sciences, Georg-August-Universität Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany. ³Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany.

*Present address: Department for Molecular Biology of Plant-Microbe Interactions, Albrecht-von-Haller Institute for Plant Sciences, Georg-August-Universität Göttingen, Untere Karspüle 2, 37073 Göttingen, Germany.

†Present address: Febit Biomed GmbH, Im Neuenheimer Feld 519, 69120 Heidelberg, Germany.

‡Present address: Bio-Protection Research Centre, Post Office Box 84, Lincoln University, Lincoln 7647, New Zealand.

§Present address: Institute for Plant Health, Austrian Agency for Health and Food Safety (AGES), Spargelfeldstraße 191, 1226 Wien, Austria.

||Present address: Roy J. Carver Center for Comparative Genomics, University of Iowa, Department of Biological Sciences, 303 Biology Building, Iowa City, IA 52242, USA.

¶Present address: Research School of Biology, Building 41, Linnaeus Way, Australian National University, Canberra, ACT 0200, Australia.

#To whom correspondence should be addressed. E-mail: Kahmann@mpi-marburg.mpg.de

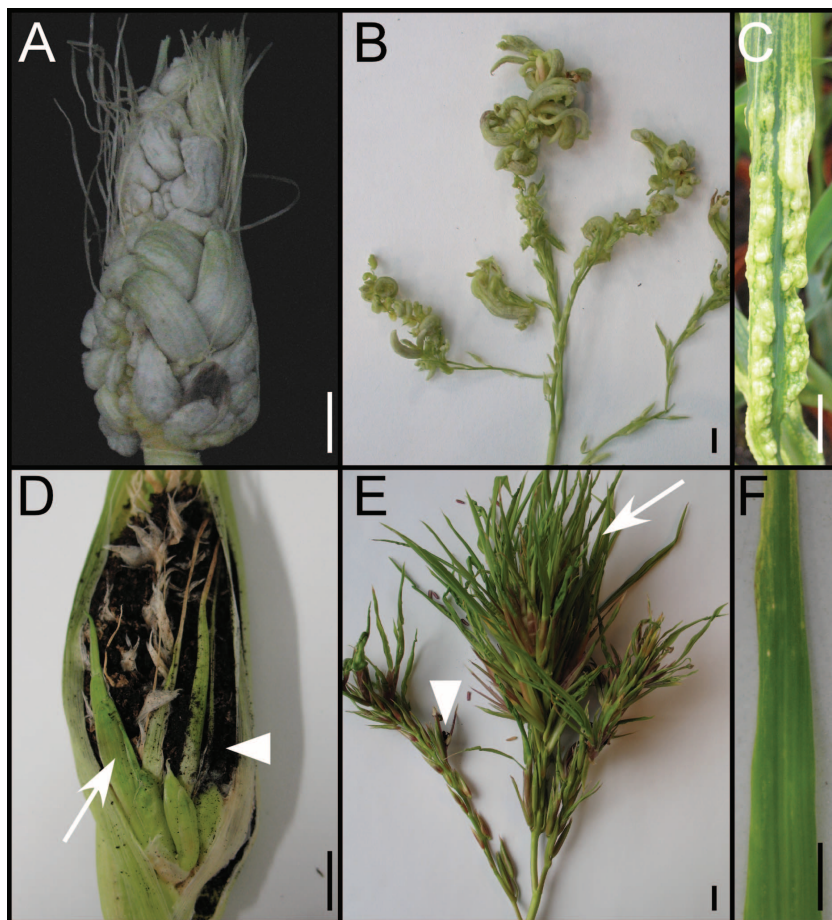


Fig. 1. Infection symptoms of *U. maydis* and *S. reilianum*. Tumor formation on ear (A), tassel (B), and leaf (C) after inflorescence infection [(A) and (B)] or seedling infection (C) by *U. maydis*. Maize seedling infection with *S. reilianum* leads to formation of spores (arrowheads) and leaflike structures (arrows) in ear (D) and tassel (E), whereas inoculated leaves show mild chlorosis but no tumors (F). Scale bars indicate 1 cm.

respective genes. Instead, we detected between one and four variants of a conserved 10-base pair (bp) sequence highly overrepresented in intergenic regions of *U. maydis* (3) that is largely absent from the genome of *S. reilianum* (table S4). We speculate that the genes encoding components of the RNAi machinery were lost in *U. maydis* by rare homologous recombination events involving the 10-bp motifs. To investigate whether the generation of small regulatory RNAs could explain the differences in symptoms of *U. maydis* and *S. reilianum*, we deleted the putative dicer gene *sr16838* in a solopathogenic strain of *S. reilianum* (8). Infection experiments revealed that *sr16838* deletion mutants were affected in neither virulence nor symptom development (fig. S2).

To detect regions of high sequence divergence, we compared the genomes of *U. maydis* and *S. reilianum* gene by gene (8, 10) and identified regions encoding genes with low sequence conservation ("divergence clusters") in a conserved genomic context (8). This analysis revealed the presence of 43 divergence clusters (table S5) (10). Seventy-one percent of the genes in divergence clusters occurred in both organisms, whereas 19% were *S. reilianum*-specific and 10% were *U. maydis*-specific. Sixty-one percent of the genes in divergence clusters are predicted to encode secreted proteins. In contrast, of all *S. reilianum* and *U. maydis* genes less than 12% encode potentially secreted proteins. Ninety-four of the genes in divergence clusters code for proteins without functional annotation (table S5). It is notable that 442 of the 494 putative effectors detected in *U. maydis* are conserved in *S. reilianum*, with amino acid identities ranging from 20 to 98% (10). In addition, 445 genes are present only in *U. maydis*, whereas 372 exist only in *S. reilianum*, and of these about 15% encode secreted proteins.

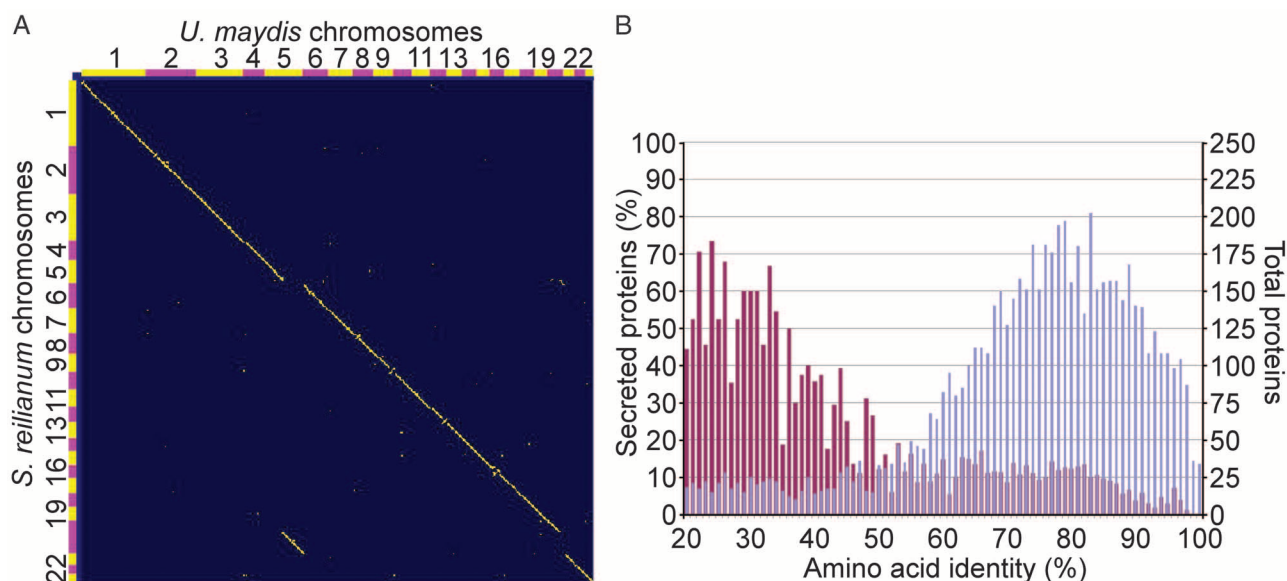


Fig. 2. Comparison of *U. maydis* and *S. reilianum* genomes. (A) Synteny (diagonal lines) of protein-coding genes on the 23 chromosomes of *U. maydis* compared to those on the 23 chromosomes of *S. reilianum*. (B) Distribution of

amino acid identities of all protein-coding genes occurring in both genomes (right axis, blue bars). The percentage of proteins with a predicted secretion signal is given for each amino acid identity value (left axis, red bars).

Among the divergence gene clusters identified in this study were 7 of the 12 previously described *U. maydis* effector gene clusters (3), and these included all clusters whose deletion affected virulence in seedling infections (3) (fig. S3). In contrast, four of the gene clusters of secreted proteins without a deletion phenotype in *U. maydis* seedling infections (3) did not classify as divergence clusters (fig. S4). To test whether any of the newly identified divergence gene clusters also harbor virulence factors, we individually deleted six randomly chosen divergence clusters in *U. maydis* (Fig. 3). In seedling infection assays, loss of three divergence gene clusters (15-12, 5-21, and 20-15) attenuated virulence, whereas deletion of two clusters (5-18 and 11-16) did not affect virulence (Fig. 3 and fig. S5). The absence of a virulence phenotype likely reflects redundancy because in these cases potential paralogs exist elsewhere in the genome (table S6). In cluster 8-12, we detected the *mig1* gene that is highly induced during plant colonization and encodes an effector with similarity to apoplast fungal avirulence proteins (13, 14). Cluster 8-12 contains three additional genes, of which two encode proteins with similarity to Mig1. In *S. reilianum*, the *mig1* gene family is expanded to

eight members residing in a single cluster (Fig. 3 and fig. S6). Whereas the deletion of only *mig1* did not affect virulence (13), cluster 8-12 deletion caused hypervirulence (fig. S5). Hypervirulence could result from an active attenuation of fungal proliferation by respective effectors (3). However, given the conserved features between avirulence proteins and Mig1 effectors, we now propose that genes whose deletion leads to hypervirulence encode weak avirulence proteins that trigger defense responses in plants expressing a cognate resistance protein, resulting in an attenuation of fungal growth.

Although most of the deleted genes in the four divergence clusters with an effect on virulence encode putatively secreted proteins, cluster 15-12 encodes only proteins without identifiable secretion signals, suggesting that additional mechanisms for virulence modulation exist in *U. maydis*. With respect to the origin of these clusters, we do not detect hallmarks for horizontal gene transfer like an altered GC content or an association with repetitive elements (fig. S7). Therefore, because many divergence regions contain members of small gene families (table S4) we propose that the majority of divergence clusters have been generated by local gene duplications followed by strong natural se-

lection resulting from interaction with different host molecules.

Our studies demonstrate the power of comparative genomics of closely related species for the identification of new virulence genes. The *U. maydis* and *S. reilianum* pathosystems present a unique example of differentiation of two closely related pathogens parasitizing the same host. We have recognized that the *U. maydis* and *S. reilianum* genomes comprise conserved effector genes as expected for pathogens infecting the same host. However, although the two pathogens are both recognized and challenged by the maize immune system, they also possess strongly differentiated effectors, suggesting that they are targeting different host molecules. We speculate that their different infection strategies lead to the interaction between different host-pathogen molecules and thereby the evolution of differentiated sets of effector proteins, although we cannot exclude contributions of the species-specific genes. The assertion that closely related pathogens interact with and affect different host targets suggests a high variety in pathogen targets within the same host. It also suggests a temporal and spatial difference in the composition of different host proteins, which can drive the evolution of different sets of effectors in pathogens with different infection strategies.

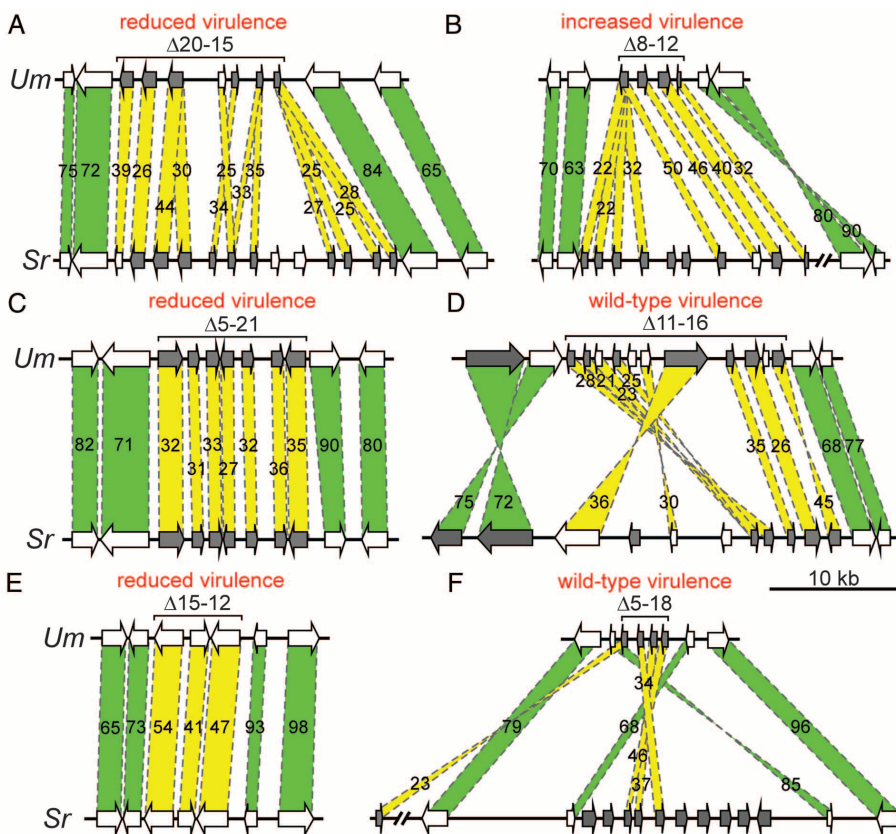


Fig. 3. Gene-by-gene comparisons of divergence clusters between *U. maydis* (Um) and *S. reilianum* (Sr) deleted in this study. (A) Cluster 15-12, (B) cluster 20-15, (C) cluster 11-16, (D) cluster 8-12, (E) cluster 5-21, and (F) cluster 5-18. Genes encoding putatively secreted proteins are shaded in gray. Bars connecting syntenic homologs are color-coded (green, high; yellow, weak conservation), and numbers give amino acid identities. Brackets denote regions deleted in *U. maydis* mutants. Virulence phenotypes of the respective mutants are indicated; the corresponding scores are found in fig. S5.

References and Notes

1. M. Stoll, D. Begerow, F. Oberwinkler, *Mycol. Res.* **109**, 342 (2005).
2. C. Martinez, C. Roux, R. Dargent, *Phytopathology* **89**, 247 (1999).
3. J. Kämper et al., *Nature* **444**, 97 (2006).
4. T. Brefort et al., *Annu. Rev. Phytopathol.* **47**, 423 (2009).
5. G. Doehlemann et al., *PLoS Pathog.* **5**, e1000290 (2009).
6. D. S. Skibbe, G. Doehlemann, J. Fernandes, V. Walbot, *Science* **328**, 89 (2010).
7. B. J. Haas et al., *Nature* **461**, 393 (2009).
8. Materials and methods are available as supporting material on Science Online.
9. MIPS, *Sporisorium reilianum* data base (MSRDB), <http://mips.helmholtz-muenchen.de/genre/proj/sporisorium>.
10. http://mips.helmholtz-muenchen.de/genre/export/sites/default/sporisorium/Download/Genome_comparison.xls
11. E. Bernstein, A. A. Caudy, S. M. Hammond, G. J. Hannon, *Nature* **409**, 363 (2001).
12. C. Catalanotto, G. Azzalini, G. Macino, C. Cogoni, *Nature* **404**, 245 (2000).
13. C. W. Basse, S. Stumpferl, R. Kahmann, *Mol. Cell. Biol.* **20**, 329 (2000).
14. I. Stergiopoulos, P. J. de Wit, *Annu. Rev. Phytopathol.* **47**, 233 (2009).
15. We are grateful to the Max Planck Society for funding genome sequencing and annotation. T.B. was supported through the Deutsche Forschungsgemeinschaft via SFB593. A.M.M. received a Humboldt fellowship. H.G. was funded by the International Max Planck Research School for Environmental, Cellular, and Molecular Microbiology. R.K. acknowledges support through the LOEWE program of the State of Hesse. We thank U. Bonas and G. Gottschalk for critical comments on the manuscript. The sequence was submitted to European Molecular Biology Laboratory database under accession numbers FQ311430 to FQ311474.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1546/DC1
Materials and Methods
Figs. S1 to S7
Tables S1 to S6
References

19 July 2010; accepted 25 October 2010
10.1126/science.1195330

Signatures of Adaptation to Obligate Biotrophy in the *Hyaloperonospora arabidopsidis* Genome

Laura Baxter,^{1*†} Sucheta Tripathy,^{2*} Naveed Ishaque,^{3‡} Nico Boot,⁴ Adriana Cabral,⁴ Eric Kemen,³ Marco Thines,^{3,5,6} Audrey Ah-Fong,⁷ Ryan Anderson,⁸ Wole Badejoko,¹ Peter Bittner-Eddy,^{1†} Jeffrey L. Boore,⁹ Marcus C. Chibucos,^{2†} Mary Coates,¹ Paramvir Dehal,¹⁰ Kim Delehaunty,¹¹ Suomeng Dong,^{12,13} Polly Downton,^{1†} Bernard Dumas,^{14,15} Georgina Fabro,³ Catrina Fronick,¹¹ Susan I. Fuerstenberg,⁹ Lucinda Fulton,¹¹ Elodie Gaulin,^{14,15} Francine Govers,¹⁶ Linda Hughes,¹ Sean Humphray,¹⁷ Rays H. Y. Jiang,^{16,18} Howard Judelson,⁷ Sophien Kamoun,³ Kim Kyung,¹¹ Harold Meijer,¹⁶ Patrick Minx,¹¹ Paul Morris,¹⁹ Joanne Nelson,¹¹ Vipa Phuntumart,¹⁹ Dinah Qutob,¹² Anne Rehmany,¹ Alejandra Rougon-Cardoso,^{3†} Peter Ryden,^{1†} Trudy Torto-Alalibo,² David Studholme,^{3†} Yuanchao Wang,¹³ Joe Win,³ Jo Wood,¹⁷ Sandra W. Clifton,¹¹ Jane Rogers,^{1†} Guido Van den Ackerveken,⁴ Jonathan D. G. Jones,^{3†‡} John M. McDowell,^{8‡§} Jim Beynon,^{1‡} Brett M. Tyler^{2,8‡}

Many oomycete and fungal plant pathogens are obligate biotrophs, which extract nutrients only from living plant tissue and cannot grow apart from their hosts. Although these pathogens cause substantial crop losses, little is known about the molecular basis or evolution of obligate biotrophy. Here, we report the genome sequence of the oomycete *Hyaloperonospora arabidopsidis* (*Hpa*), an obligate biotroph and natural pathogen of *Arabidopsis thaliana*. In comparison with genomes of related, hemibiotrophic *Phytophthora* species, the *Hpa* genome exhibits dramatic reductions in genes encoding (i) RXLR effectors and other secreted pathogenicity proteins, (ii) enzymes for assimilation of inorganic nitrogen and sulfur, and (iii) proteins associated with zoospore formation and motility. These attributes comprise a genomic signature of evolution toward obligate biotrophy.

The oomycete *Hyaloperonospora arabidopsidis* (*Hpa*, formerly *Peronospora parasitica* or *Hyaloperonospora parasitica*) is a natural pathogen of *Arabidopsis thaliana* and a model for dissection of *A. thaliana* pathogen response networks (1, 2). *Hpa* belongs to a group of “downy mildew” pathogens, comprising more than 800 species that cause disease on hundreds of plant species (3). Downy mildew pathogens are related to other destructive oomycete plant pathogens (e.g., *Phytophthora* species) (4, 5). Oomycetes belong to the kingdom Stramenopila, which includes brown algae and diatoms. Although oomycetes and fungi share morphological and ecological similarities, they evolved independently to colonize plants.

Hpa hyphae grow between plant cells and establish feeding structures called haustoria, which have also evolved in fungal pathogens (2, 6). Downy mildews are obligately biotrophic and cannot be cultured apart from their hosts. In contrast, *Phytophthora* species are hemibiotrophic; an initial phase of biotrophic growth is followed by a necrotrophic phase. Molecular phylogenies show that downy mildews arose from a paraphyletic, *Phytophthora*-like, hemibiotrophic ancestor (4, 5, 7). Thus, insight into the genomic basis and evolution of obligate biotrophy can be obtained through comparison of the *Hpa* genome to the recently sequenced genomes of *Phytophthora* species (8, 9).

Genome analysis was performed on the *Hpa* Emoy2 isolate (1) using DNA from asexual spores (10). Sanger shotgun sequencing at 9.5-fold cov-

erage, combined with 97 sequenced bacterial artificial chromosome inserts, yielded an assembly of 77.8 Mb. Illumina sequencing at 46-fold coverage yielded 3.8 Mb of additional sequence, which was integrated into the Sanger assembly to form the 81.6-Mb final version (v8.3.2). Forty-two percent of the *Hpa* genome is composed of repetitive elements (table S1). Analysis of Sanger and Illumina read depth suggests that v8.3 contains ~12.7 Mb composed of tandem repeats compressed into reduced copies in the assembly, indicative of a genome size of around 100 Mb (fig. S1). The CEGMA (Core Eukaryotic Genes Mapping Approach) pipeline, combined with manual exami-

nation, identified *Hpa* orthologs of 95% of the 248 conserved single-copy eukaryotic genes (fig. S2). Moreover, 94% of 31,759 expressed sequence tag (EST) reads aligned to the assembly, indicating that the draft genome assembly encompasses a very high percentage of the *Hpa* gene space.

A total of 14,543 genes were computationally predicted in v8.3, of which 80% are supported by ESTs and/or Illumina cDNA tags. This predicted gene content is similar to *P. ramorum* (65 Mb, 15,743 genes) and lower than *P. sojae* (95 Mb, 19,027 genes) (9) or *P. infestans* (240 MB,

¹School of Life Sciences, Warwick University, Wellesbourne, CV35 9EF, UK. ²Virginia Bioinformatics Institute, Virginia Polytechnic Institute, Blacksburg, VA 24061, USA. ³Sainsbury Laboratory, John Innes Centre, Norwich NR4 7UH, UK. ⁴Plant-Microbe Interactions, Department of Biology, Utrecht University, Padualaan 8, 3584 CH, Utrecht, Netherlands. ⁵Biodiversity and Climate Research Centre (BiK-F), Senckenberganlage 25, D-60325 Frankfurt (Main), Germany. ⁶Johann Wolfgang Goethe University, Department of Biological Sciences, Institute of Ecology, Evolution and Diversity, Siesmayerstr. 70, D-60323 Frankfurt (Main), Germany. ⁷Department of Plant Pathology and Microbiology, University of California, Riverside, CA 92521, USA. ⁸Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute, Blacksburg, VA 24061, USA. ⁹Department of Integrative Biology, University of California, Berkeley, USA. ¹⁰Lawrence Berkeley National Laboratories, Berkeley, CA 94720, USA. ¹¹Genome Sequencing Centre, Washington University School of Medicine, St. Louis, MO 63110, USA. ¹²Agriculture and Agri-Food Canada, London, Ontario N5V 4T3, Canada. ¹³Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China. ¹⁴Université de Toulouse, UPS, Surfaces Cellulaires et Signalisation chez les Végétaux, 24 chemin de Borde Rouge, BP42617, Auzerville, F-31326 Castanet-Tolosan, France. ¹⁵CNRS, Surfaces Cellulaires et Signalisation chez les Végétaux, 24 chemin de Borde Rouge, BP42617, Auzerville, F-31326 Castanet-Tolosan, France. ¹⁶Laboratory of Phytopathology, Wageningen University, and Centre for BioSystems Genomics, NL-1-6708 PB Wageningen, Netherlands. ¹⁷Sanger, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK. ¹⁸The Broad Institute of MIT and Harvard, Cambridge, MA 02141–2023, USA. ¹⁹Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403–0212, USA.

*These authors contributed equally to this work.

†Present addresses and other affiliations are listed in the supporting online material.

‡These authors contributed equally to this work.

§To whom correspondence should be addressed. E-mail: johnmcd@vt.edu

Table 1. Copy numbers of annotated *Hpa* genes for hydrolases, PAMPS, and effectors, compared with *Phytophthora* genomes.

Gene product	<i>H. arabidopsidis</i>	<i>P. sojae</i>	<i>P. ramorum</i>
Extracellular proteases	18	47	48
Glycosyl hydrolases	>60	125	114
Endoglucanases (EGL12)	3	10	8
Polygalacturonases	3	25	16
Pectin methyl esterases	3	19	15
Cutinases	2	16	4
Chitinases	1	5	2
Elicitins	1	18	17
Elicitin-like	14	39	31
CBEL and CBEL-like	2	13	15
RXLR	134	396	374
NLP	10	29	40
Crinklers	20	40	8
PPAT12/24-like	8	0	0

17,887 genes) (8). A total of 6882 predicted genes in *Hpa* had no identifiable ortholog in sequenced *Phytophthora* species or similarity to known proteins, and as such represent potentially lineage-specific genes. Some of these genes may play roles that are specific to biotrophy. For example, a novel family of secreted small, cysteine-rich proteins exists in *Hpa* (PPAT12/24-like) (Table 1) (11).

Pathogenicity genes were compared among *Hpa* and *Phytophthora* species, revealing that families encoding host-targeted, degradative enzymes (secreted proteinases, cell wall-degrading enzymes) are reduced in *Hpa* (Table 1). Two notable examples are the family 12 endoglucanases (*EGL12*) and pectin methyl esterases (*Pect*). Phylogenetic analyses delineated several *EGL12* and *Pect* gene clades containing genes from *P. sojae* and *P. ramorum* but not *Hpa* (figs. S6 and S7). Because *Hpa* and *P. sojae* likely share a sister group relationship relative to *P. ramorum* (4, 5), it is probable that a number of *EGL12* and *Pect* genes were lost from *Hpa* after divergence of the lineage leading to *Hpa* and *P. sojae*. Hydrolytic enzymes that target the host cell wall can release cell wall fragments that elicit host defenses. It is conceivable that in evolving a biotrophic lifestyle, *Hpa* has lost most of the secreted hydrolytic enzymes that were present in a hemibiotrophic ancestor.

Similarly, gene families encoding necrosis and ethylene-inducing (Nep1)-like proteins (NLPs) are significantly reduced in *Hpa*, compared with *P. sojae* and *P. ramorum*. NLPs in *Phytophthora* and *Pythium* can trigger plant cell death and defenses (12), which have been implicated in the transition from biotrophy to necrotrophy. Only three of the 13 oomycete NLP clades contain genes from *Hpa*. However, one clade contains an expanded family that is specific to *Hpa* (Fig. 1A). All 10 *HaNLP* genes are supported by transcriptional data. Of these, *HaNLP3* is most closely related to the *PsojNIP* and *PinPP1.1* proteins, but it did not induce necrosis in *Nicotiana tabacum* (Fig. 1B). These results suggest that downy mildew NLP genes may have evolved a different function than in *Phytophthora*. Copy number reduction was also evident for genes encoding known pathogen-associated molecular patterns (PAMPs) such as sterol-binding elicitors (13) and carbohydrate-binding CBEL (cellulose-binding, elicitor, lectin-like) genes (14) (Table 1). These examples further suggest that selection for “stealth” (avoidance of host defenses) was a major force during downy mildew evolution.

Phytophthora genomes encode hundreds of potential effector proteins (9, 15, 16) with RXLR cell entry motifs (16–18) that likely function to suppress host defenses (19, 20). The *Hpa* genome contained 134 high-confidence effector gene candidates (*HaRxL* genes), including the known effector genes *Atr1* and *Atr13* (21, 22), significantly fewer than in the *Phytophthora* genomes (9, 15). Single-nucleotide polymorphisms arising from heterozygosity in v8.3 occurred at a

rate 5 times as high in RXLR effector candidates [1 per ~500 base pairs (bp)] than in other genes (1 per ~2500 bp). Only 36% of the high-confidence

Hpa effectors had significant matches in any *Phytophthora* genome (sequence similarity >30%), consistent with strong divergent selection on

Fig. 1. Diversity, evolutionary history, and functional analysis of oomycete necrosis and NLPs. (A) Phylogeny of oomycete NLPs. A consensus tree from the Bayesian inference is shown. Thick lines indicate high support in minimum evolution (>90), maximum likelihood (>90), and Bayesian inference (>0.95). Hollow lines indicate branches highly supported in at least two analyses. Branches with high support in less than two analyses are represented by thin lines. (B) An *Hpa* NLP ortholog does not induce necrosis in plant leaves. NLP genes were transiently expressed in *Nicotiana tabacum* by agroinfiltration.

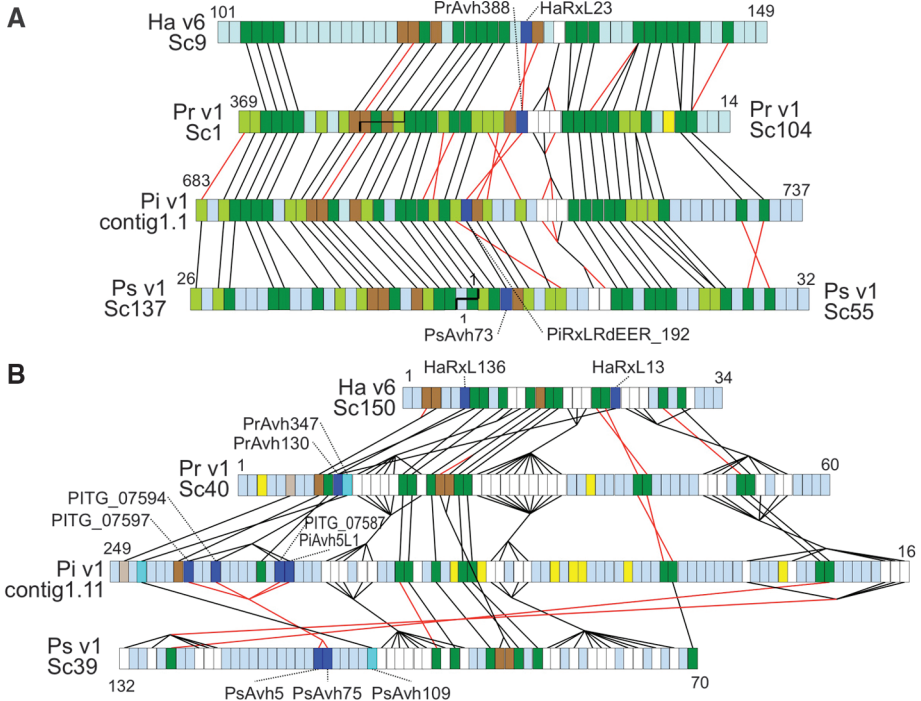
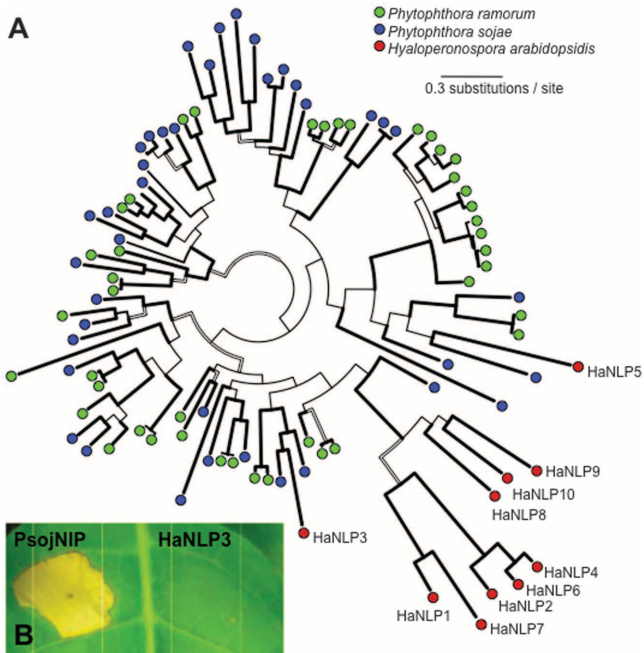


Fig. 2. Synteny of conserved RXLR effectors. (A) Region around *HaRxL23*, spanning scaffold_9:467737-739923 (v6) and supercontig16:325445-40488 (v8.3.2) (B) Region around *HaRxL13* and *HaRxL136*, spanning scaffold_150:3503-183330 (v6) and supercontig35:456072-268293 (v8.3.2). Colored boxes show order of gene models. Noncoding DNA is not represented. Dark green, orthologs; light green, orthologs found only in *Phytophthora*; dark brown, syntenic paralogs; light brown, syntenic orthologs found only in *Phytophthora*; white, syntenic gene families; dark blue, syntenic conserved RXLR effectors; cyan, syntenic RXLR effectors conserved only in *Phytophthora*; yellow, RXLR effectors not syntenic or conserved; blue-gray, other genes not conserved or syntenic. Black lines join syntenic genes with the same orientation; red lines join genes with reversed orientations. Staggered black lines in (A) show scaffold joins predicted from the synteny analysis. *HaRxL23*, *HaRxL13*, and *HaRxL136* have 47, 38, and 40% amino acid identity, respectively, with their most similar *Phytophthora* ortholog within the normally hypervariable C terminus.

RXLR effector genes (15, 23). Moreover, *Hpa* effectors generally were not located in syntenic locations relative to *Phytophthora* genomes, except for three families of effectors, which have unusually high levels of sequence conservation (Fig. 2).

As obligate biotrophs, downy mildews may have lost some metabolic pathways. We identified several potential metabolic defects in *Hpa* compared with *P. sojae* and *P. ramorum* (fig. S9). For example, genes for nitrate and nitrite reductases, a nitrate transporter, and sulfite reductase were missing (fig. S10 and table S3), which is also a feature of the genomes of obligately parasitic powdery mildew fungi (24). *Hpa* also lacks genes required for synthesis of arachidonic acid and polyamine oxidases.

Flagellated zoospores are produced by many oomycetes (25). Contrastingly, several downy mildew lineages germinate by extending infective germ tubes from nonmotile conidiospores, although evidence exists for a rare zoospore stage in some otherwise conidial downy mildews (26, 27). To conclusively determine whether spore motility has been lost from the *Hpa* lineage, we searched the *Hpa* genome for 90 flagella-associated genes using *Chlamydomonas* sequences and their *Phytophthora* orthologs (28). No matches were detected in *Hpa* for any of these. Similarly, many *Phytophthora* adhesion-related genes are reduced in number or absent from *Hpa*, consistent with the lack of adherent cysts that normally develop from zoospores during infection.

Analysis of *Hpa* gene space revealed genomic signatures of major alterations in pathogenic strategy, metabolism, and development that occurred

during the evolution of obligate biotrophy from a facultative, hemibiotrophic ancestor. Interestingly, some features of *Hpa* gene space (large numbers of secreted effectors, reduction in degradative enzymes, and loss of N and S assimilation) are mirrored in genomes of biotrophic fungi (24, 29, 30). These similarities indicate that convergent adaptations occurred during the independent evolution of biotrophy in fungal and oomycete lineages.

References and Notes

1. E. B. Holub, *Eur. J. Plant Pathol.* **122**, 91 (2008).
2. M. E. Coates, J. L. Beynon, *Annu. Rev. Phytopathol.* **48**, 329 (2010).
3. J. Clark, P. Spencer-Phillips, in *Encyclopedia of Microbiology* (Academic Press, 2000), vol. 2, pp. 117–129.
4. X. Giresse, S. Ahmed, S. Richard-Cervera, F. Delmotte, *J. Phytopathol.* **158**, 321 (2010).
5. M. Göker, H. Voglmayr, A. Riethmüller, F. Oberwinkler, *Fungal Genet. Biol.* **44**, 105 (2007).
6. R. Panstruga, P. N. Dodds, *Science* **324**, 748 (2009).
7. M. Thines, *PLoS ONE* **4**, e4790 (2009).
8. B. J. Haas et al., *Nature* **461**, 393 (2009).
9. B. M. Tyler et al., *Science* **313**, 1261 (2006).
10. Materials and methods are available as supporting material on Science Online.
11. P. D. Bittner-Eddy, R. L. Allen, A. P. Rehmany, P. Birch, J. L. Beynon, *Mol. Plant Pathol.* **4**, 501 (2003).
12. M. Gijzen, T. Nürnberger, *Phytochemistry* **67**, 1800 (2006).
13. S. Kamoun, *Annu. Rev. Phytopathol.* **44**, 41 (2006).
14. E. Gaulin et al., *Plant Cell* **18**, 1766 (2006).
15. R. H. Jiang, S. Tripathy, F. Govers, B. M. Tyler, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 4874 (2008).
16. S. C. Whisson et al., *Nature* **450**, 115 (2007).
17. D. Dou et al., *Plant Cell* **20**, 1930 (2008).
18. S. Kale et al., *Cell* **142**, 284 (2010).
19. D. Dou et al., *Plant Cell* **20**, 1118 (2008).
20. K. H. Sohn, R. Lei, A. Nemri, J. D. Jones, *Plant Cell* **19**, 4077 (2007).
21. R. L. Allen et al., *Science* **306**, 1957 (2004).
22. A. P. Rehmany et al., *Plant Cell* **17**, 1839 (2005).
23. J. Win et al., *Plant Cell* **19**, 2349 (2007).
24. P. Spanu, *Science* **330**, 1543 (2010).
25. A. R. Hardham, G. J. Hyde, *Adv. Bot. Res.* **24**, 353 (1997).
26. D. G. Milbrath, *J. Agric. Sci.* **23**, 989 (1923).
27. V. Skalicky, *Preslia* **38**, 117 (1966).
28. G. J. Pazour, N. Agrin, J. Leszyk, G. B. Witman, *J. Cell Biol.* **170**, 103 (2005).
29. J. Kämper et al., *Nature* **444**, 97 (2006).
30. F. Martin et al., *Nature* **452**, 88 (2008).
31. We thank E. Holub for providing the Emoy2 isolate, D. Greenshields and N. Bruce for technical assistance, A. Heck and M. Slijper for analysis of secreted *Hpa* proteins, R. Hubley for creating repeat modeller libraries, and participants in the 2007 Annotation Jamboree and in the 2008 and 2009 Oomycete Bioinformatics Training Workshops for sequence annotations. This research was supported by grants EF-0412213, IOS-0744875, IOS-0924861, and MCB-0639226 from the U.S. NSF and 2004-35600-15055 and 2007-35319-18100 from the U.S. Department of Agriculture National Institute of Food and Agriculture to B.M.T. and J.M.M.; Biotechnology and Biological Sciences Research Council (BBSRC) BB/C509123/1, BB/E024815/1, and Engineering and Physical Sciences Research Council/BBSRC Systems Biology DTC student EP/F500025/1 to J.B.; Gatsby GAT2545 and BBSRC BB/F0161901, BB/E024882/1, and BBSRC CASE studentship T12144 to J.D.G.J. Other support is detailed in the supporting online material. Genome browsers are maintained at the Virginia Bioinformatics Institute (vmd.vbi.vt.edu) and the Sainsbury Laboratories (gbrowse2.tsl.ac.uk/cgi-bin/gb2/gbrowse/hpa_emoy2_publication).

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1549/DC1
Materials and Methods

Figs. S1 to S10

Tables S1 to S3

References

16 July 2010; accepted 25 October 2010
10.1126/science.1195203

The Major Genetic Determinants of HIV-1 Control Affect HLA Class I Peptide Presentation

The International HIV Controllers Study*†

Infectious and inflammatory diseases have repeatedly shown strong genetic associations within the major histocompatibility complex (MHC); however, the basis for these associations remains elusive. To define host genetic effects on the outcome of a chronic viral infection, we performed genome-wide association analysis in a multiethnic cohort of HIV-1 controllers and progressors, and we analyzed the effects of individual amino acids within the classical human leukocyte antigen (HLA) proteins. We identified >300 genome-wide significant single-nucleotide polymorphisms (SNPs) within the MHC and none elsewhere. Specific amino acids in the *HLA-B* peptide binding groove, as well as an independent *HLA-C* effect, explain the SNP associations and reconcile both protective and risk *HLA* alleles. These results implicate the nature of the HLA–viral peptide interaction as the major factor modulating durable control of HIV infection.

HIV infection is characterized by acute viremia, often in excess of 5 million viral particles per milliliter of plasma, followed by an average 100-fold or greater decline to a relatively stable plasma virus load set point (1). In the absence of antiretroviral therapy, the

level of viremia is associated with the rate of CD4⁺ T cell decline and progression to AIDS. There is substantial interperson variability in the virus load set point, with most individuals having stable levels exceeding 10,000 RNA copies/ml. Yet a small number of people demonstrate sus-

tained ability to control HIV replication without therapy. Such individuals, referred to as HIV controllers, typically maintain stable CD4⁺ cell counts, do not develop clinical disease, and are less likely to transmit HIV to others (2).

To determine the genetic basis for this rare phenomenon, we established a multinational consortium (www.hivcontrollers.org) to recruit HIV-1 controllers, who are defined by at least three measurements of plasma virus load (VL) < 2000 RNA copies/ml over at least a 12-month period in the absence of antiviral therapy. We performed a genome-wide association study (GWAS) in the HIV controllers (median VL, CD4 count, and disease duration of 241 copies/ml, 699 cells/mm³, and 10 years, respectively) and treatment-naïve chronically infected individuals with advanced disease (median VL and CD4 count of 61,698 copies/ml and 224 cells/mm³, respectively) enrolled in antiviral treatment studies led by the AIDS Clinical Trials Group. After quality control and imputation on the basis of HapMap

*All authors with their contributions and affiliations appear at the end of this paper.

†To whom correspondence should be addressed. E-mail: bwalker@partners.org (B.D.W.); pdebakker@rics.bwh.harvard.edu (P.I.W.d.B.)

RXLR effector genes (15, 23). Moreover, *Hpa* effectors generally were not located in syntenic locations relative to *Phytophthora* genomes, except for three families of effectors, which have unusually high levels of sequence conservation (Fig. 2).

As obligate biotrophs, downy mildews may have lost some metabolic pathways. We identified several potential metabolic defects in *Hpa* compared with *P. sojae* and *P. ramorum* (fig. S9). For example, genes for nitrate and nitrite reductases, a nitrate transporter, and sulfite reductase were missing (fig. S10 and table S3), which is also a feature of the genomes of obligately parasitic powdery mildew fungi (24). *Hpa* also lacks genes required for synthesis of arachidonic acid and polyamine oxidases.

Flagellated zoospores are produced by many oomycetes (25). Contrastingly, several downy mildew lineages germinate by extending infective germ tubes from nonmotile conidiospores, although evidence exists for a rare zoospore stage in some otherwise conidial downy mildews (26, 27). To conclusively determine whether spore motility has been lost from the *Hpa* lineage, we searched the *Hpa* genome for 90 flagella-associated genes using *Chlamydomonas* sequences and their *Phytophthora* orthologs (28). No matches were detected in *Hpa* for any of these. Similarly, many *Phytophthora* adhesion-related genes are reduced in number or absent from *Hpa*, consistent with the lack of adherent cysts that normally develop from zoospores during infection.

Analysis of *Hpa* gene space revealed genomic signatures of major alterations in pathogenic strategy, metabolism, and development that occurred

during the evolution of obligate biotrophy from a facultative, hemibiotrophic ancestor. Interestingly, some features of *Hpa* gene space (large numbers of secreted effectors, reduction in degradative enzymes, and loss of N and S assimilation) are mirrored in genomes of biotrophic fungi (24, 29, 30). These similarities indicate that convergent adaptations occurred during the independent evolution of biotrophy in fungal and oomycete lineages.

References and Notes

1. E. B. Holub, *Eur. J. Plant Pathol.* **122**, 91 (2008).
2. M. E. Coates, J. L. Beynon, *Annu. Rev. Phytopathol.* **48**, 329 (2010).
3. J. Clark, P. Spencer-Phillips, in *Encyclopedia of Microbiology* (Academic Press, 2000), vol. 2, pp. 117–129.
4. X. Giresse, S. Ahmed, S. Richard-Cervera, F. Delmotte, *J. Phytopathol.* **158**, 321 (2010).
5. M. Göker, H. Voglmayr, A. Riethmüller, F. Oberwinkler, *Fungal Genet. Biol.* **44**, 105 (2007).
6. R. Panstruga, P. N. Dodds, *Science* **324**, 748 (2009).
7. M. Thines, *PLoS ONE* **4**, e4790 (2009).
8. B. J. Haas et al., *Nature* **461**, 393 (2009).
9. B. M. Tyler et al., *Science* **313**, 1261 (2006).
10. Materials and methods are available as supporting material on Science Online.
11. P. D. Bittner-Eddy, R. L. Allen, A. P. Rehmany, P. Birch, J. L. Beynon, *Mol. Plant Pathol.* **4**, 501 (2003).
12. M. Gijzen, T. Nürnberger, *Phytochemistry* **67**, 1800 (2006).
13. S. Kamoun, *Annu. Rev. Phytopathol.* **44**, 41 (2006).
14. E. Gaulin et al., *Plant Cell* **18**, 1766 (2006).
15. R. H. Jiang, S. Tripathy, F. Govers, B. M. Tyler, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 4874 (2008).
16. S. C. Whisson et al., *Nature* **450**, 115 (2007).
17. D. Dou et al., *Plant Cell* **20**, 1930 (2008).
18. S. Kale et al., *Cell* **142**, 284 (2010).
19. D. Dou et al., *Plant Cell* **20**, 1118 (2008).
20. K. H. Sohn, R. Lei, A. Nemri, J. D. Jones, *Plant Cell* **19**, 4077 (2007).
21. R. L. Allen et al., *Science* **306**, 1957 (2004).
22. A. P. Rehmany et al., *Plant Cell* **17**, 1839 (2005).
23. J. Win et al., *Plant Cell* **19**, 2349 (2007).
24. P. Spanu, *Science* **330**, 1543 (2010).
25. A. R. Hardham, G. J. Hyde, *Adv. Bot. Res.* **24**, 353 (1997).
26. D. G. Milbrath, *J. Agric. Sci.* **23**, 989 (1923).
27. V. Skalicky, *Preslia* **38**, 117 (1966).
28. G. J. Pazour, N. Agrin, J. Leszyk, G. B. Witman, *J. Cell Biol.* **170**, 103 (2005).
29. J. Kämper et al., *Nature* **444**, 97 (2006).
30. F. Martin et al., *Nature* **452**, 88 (2008).
31. We thank E. Holub for providing the Emoy2 isolate, D. Greenshields and N. Bruce for technical assistance, A. Heck and M. Slijper for analysis of secreted *Hpa* proteins, R. Hubley for creating repeat modeller libraries, and participants in the 2007 Annotation Jamboree and in the 2008 and 2009 Oomycete Bioinformatics Training Workshops for sequence annotations. This research was supported by grants EF-0412213, IOS-0744875, IOS-0924861, and MCB-0639226 from the U.S. NSF and 2004-35600-15055 and 2007-35319-18100 from the U.S. Department of Agriculture National Institute of Food and Agriculture to B.M.T. and J.M.M.; Biotechnology and Biological Sciences Research Council (BBSRC) BB/C509123/1, BB/E024815/1, and Engineering and Physical Sciences Research Council/BBSRC Systems Biology DTC student EP/F500025/1 to J.B.; Gatsby GAT2545 and BBSRC BB/F0161901, BB/E024882/1, and BBSRC CASE studentship T12144 to J.D.G.J. Other support is detailed in the supporting online material. Genome browsers are maintained at the Virginia Bioinformatics Institute (vmd.vbi.vt.edu) and the Sainsbury Laboratories (gbrowse2.tsl.ac.uk/cgi-bin/gb2/gbrowse/hpa_emoy2_publication).

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6010/1549/DC1
Materials and Methods

Figs. S1 to S10

Tables S1 to S3

References

16 July 2010; accepted 25 October 2010
10.1126/science.1195203

The Major Genetic Determinants of HIV-1 Control Affect HLA Class I Peptide Presentation

The International HIV Controllers Study*†

Infectious and inflammatory diseases have repeatedly shown strong genetic associations within the major histocompatibility complex (MHC); however, the basis for these associations remains elusive. To define host genetic effects on the outcome of a chronic viral infection, we performed genome-wide association analysis in a multiethnic cohort of HIV-1 controllers and progressors, and we analyzed the effects of individual amino acids within the classical human leukocyte antigen (HLA) proteins. We identified >300 genome-wide significant single-nucleotide polymorphisms (SNPs) within the MHC and none elsewhere. Specific amino acids in the *HLA-B* peptide binding groove, as well as an independent *HLA-C* effect, explain the SNP associations and reconcile both protective and risk *HLA* alleles. These results implicate the nature of the HLA–viral peptide interaction as the major factor modulating durable control of HIV infection.

HIV infection is characterized by acute viremia, often in excess of 5 million viral particles per milliliter of plasma, followed by an average 100-fold or greater decline to a relatively stable plasma virus load set point (1). In the absence of antiretroviral therapy, the

level of viremia is associated with the rate of CD4⁺ T cell decline and progression to AIDS. There is substantial interperson variability in the virus load set point, with most individuals having stable levels exceeding 10,000 RNA copies/ml. Yet a small number of people demonstrate sus-

tained ability to control HIV replication without therapy. Such individuals, referred to as HIV controllers, typically maintain stable CD4⁺ cell counts, do not develop clinical disease, and are less likely to transmit HIV to others (2).

To determine the genetic basis for this rare phenomenon, we established a multinational consortium (www.hivcontrollers.org) to recruit HIV-1 controllers, who are defined by at least three measurements of plasma virus load (VL) < 2000 RNA copies/ml over at least a 12-month period in the absence of antiviral therapy. We performed a genome-wide association study (GWAS) in the HIV controllers (median VL, CD4 count, and disease duration of 241 copies/ml, 699 cells/mm³, and 10 years, respectively) and treatment-naïve chronically infected individuals with advanced disease (median VL and CD4 count of 61,698 copies/ml and 224 cells/mm³, respectively) enrolled in antiviral treatment studies led by the AIDS Clinical Trials Group. After quality control and imputation on the basis of HapMap

*All authors with their contributions and affiliations appear at the end of this paper.

†To whom correspondence should be addressed. E-mail: bwalker@partners.org (B.D.W.); pdebakker@rics.bwh.harvard.edu (P.I.W.d.B.)

Phase 3 (3), we obtained data on 1,384,048 single-nucleotide polymorphisms (SNPs) in 974 controllers (cases) and 2648 progressors (controls) from multiple populations (table S1). After stratification into European, African American, and Hispanic ethnic groups (fig. S1), we tested each SNP for association using logistic regression, including the major principal components as covariates to correct for population substructure (4). In the largest group, comprising 1712 individuals of European ancestry, we identified 313 SNPs with genome-wide signif-

icance, defined by $P < 5 \times 10^{-8}$ due to correction for multiple comparisons (table S2). All SNPs that reached genome-wide significance were located in the major histocompatibility complex (MHC) region on chromosome 6 (Fig. 1A). We obtained similar results for the other two ethnic groups and in a meta-analysis of all participants (fig. S2). We also performed a genome-wide analysis to test the influence of local chromosomal ancestry in the African American sample (4), but we detected no signal outside the MHC (figs. S3 and S4). The impact of the MHC was further un-

derscored when we specifically tested published associations related to HIV disease progression outside the MHC. Only variants in the *CCR5-CCR2* locus—namely, *CCR5*Δ32 deletion polymorphism (5), C927T in *CCR5* (6), and Val⁶⁴→Ile⁶⁴ in *CCR2* (7)—replicate with nominal statistical significance in our study (Fig. 1B and table S3). Closer examination of the significant SNPs within the MHC showed that they are located within a 3-Mb region concentrated around class I *human leukocyte antigen (HLA)* genes (fig. S5), but extensive linkage disequilibrium (LD) makes precise assignment of causal variants challenging (8). Therefore, we used stepwise regression to define independent markers associated with host control. From the initial set of 313 SNPs that reached genome-wide significance in the European sample, for which the greatest numbers of participants were available, we found only four independent markers of association (Table 1). rs9264942, located 35 kb upstream of *HLA-C* and a putative variant associated with *HLA-C* expression levels [odds ratio (OR) = 2.9, $P = 2.8 \times 10^{-35}$, where an OR > 1 indicates a protective effect], and rs2395029, a proxy for *HLA-B*57:01* (OR = 5.3, $P = 9.7 \times 10^{-26}$), had been previously reported to be associated with virus load set point after acute infection (9). We also defined rs4418214, a noncoding SNP near *MICA* (OR = 4.4, $P = 1.4 \times 10^{-34}$), and rs3131018 in *PSORSIC3*, a gene implicated in psoriasis (OR = 2.1, $P = 4.2 \times 10^{-16}$). These four SNPs explain 19% of the observed variance of host control in the European sample; together with those in *CCR5*, these SNPs explain 23%, using Nagelkerke's approximation (Fig. 1C) (10). In the smaller African American sample, we observed 33 SNPs with genome-wide significance, four of which were identified as independent markers, but all differed from those in the European sample (Table 1). This suggests that shared causal variants are tagged by different SNPs in these two populations or that the mechanism of control differs with ethnicity. Only rs2523608 was previously identified, in a recent study of virus load set point in African Americans (11). Despite no evidence for historical recombi-

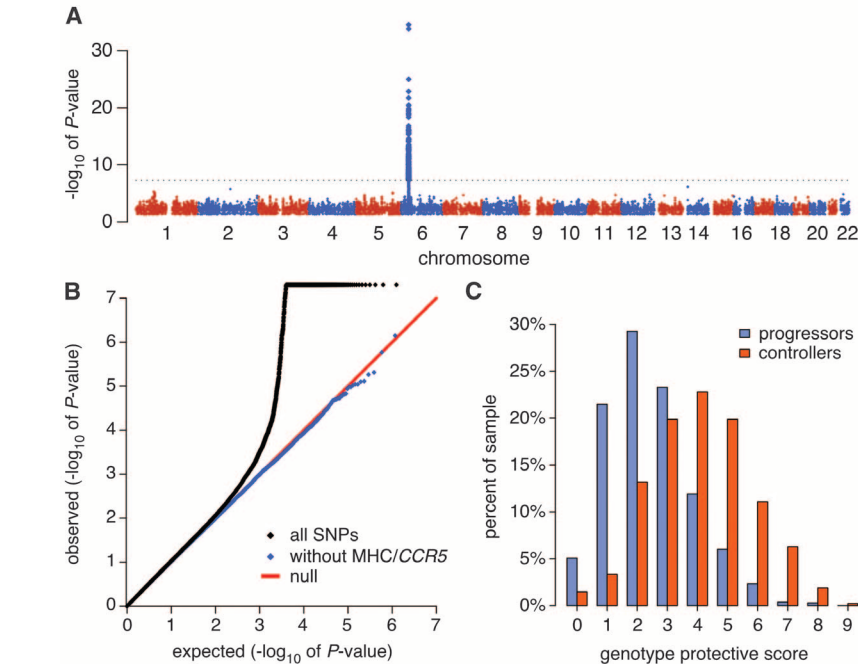


Fig. 1. Genome-wide association results in the European sample. (A) Manhattan plot of 1.3 million autosomal SNPs. Only SNPs in the MHC on chromosome 6 reach genome-wide significance, indicated by the horizontal dotted line ($P < 5 \times 10^{-8}$). Red and blue colors alternate between chromosomes. (B) Quantile-quantile plot of the association results with (black) and without (blue) SNPs in the extended MHC and the *CCR5-CCR2* locus, indicating that the detectable effect is entirely attributable to these two loci. The red line denotes the expected distribution under the null hypothesis of no effect. (C) Distribution of the genotype protective score, defined as the total number of alleles associated with host control at the four independent SNPs in the MHC and the variants at *CCR5-CCR2*, showing marked differences in controllers (orange) and progressors (blue). In aggregate, these variants explain 23% of the observed variance of durable host control.

Table 1. Association results for the independent SNPs in the MHC identified with stepwise regression in the European and African American samples. The odds ratio and frequency is given for the A1 allele, where OR >

1 indicates a protective effect. Odds ratios and *P* values were computed for univariate and multivariate regression models. C, cytosine; G, guanine; T, thymine; A, adenine.

SNP	A1	A2	Frequency in controllers	Frequency in progressors	Univariate		Multivariate	
					OR	P value	OR	P value
European								
rs9264942	C	T	0.595	0.336	2.9	2.8×10^{-35}	2.1	6.3×10^{-16}
rs4418214	C	T	0.240	0.075	4.4	1.4×10^{-34}	1.8	4.9×10^{-4}
rs2395029	G	T	0.139	0.032	5.3	9.7×10^{-26}	2.1	3.5×10^{-4}
rs3131018	C	A	0.777	0.625	2.1	4.2×10^{-16}	1.5	1.2×10^{-5}
African American								
rs2523608	G	A	0.522	0.326	2.6	8.9×10^{-20}	2.3	3.7×10^{-15}
rs2255221	T	G	0.264	0.137	2.7	3.5×10^{-14}	1.9	2.1×10^{-6}
rs2523590	C	T	0.300	0.164	2.4	1.7×10^{-13}	2.3	1.2×10^{-12}
rs9262632	G	A	0.097	0.034	3.1	1.0×10^{-8}	2.2	2.8×10^{-4}

nation ($D' = 1$), this SNP is only weakly correlated ($r^2 < 0.1$) with *HLA-B*57:03*, the class I allele most strongly associated with durable control of HIV in populations of African ancestry (11–13). In the Hispanic sample, which was much smaller, the most significant SNP was rs2523590, 2 kb upstream of *HLA-B*, also identified in the African American sample described here.

Given the localization of significant SNPs entirely to the HLA class I region, as well as previous studies showing *HLA* alleles to affect disease progression (13–20), we next sought to evaluate whether these SNP and HLA associations might be due to specific amino acids within HLA. Because *HLA* types were available for only a portion of the entire cohort, we developed a method to impute classical *HLA* alleles and their corresponding amino acid sequences (4) on the basis of haplotype patterns in an independent data set collected by the Type 1 Diabetes Genetics Consortium (T1DGC) (21). This data set contains genotype data for 639 SNPs in the MHC that overlap with genotyped SNPs in our GWAS and classical *HLA* types for class I and II loci at four-digit resolution in 2767 unrelated individuals of European descent.

We imputed *HLA* types in the European sample of our study and validated the imputations by comparing to empirical four-digit *HLA* typing data collected for class I loci in a subset ($n = 371$) of the HIV controllers. The quality of the imputations was such that the imputed and true frequencies for all *HLA* alleles in this subset were in near-perfect agreement (Fig. 2A) ($r^2 = 0.99$). Furthermore, the positive predictive value was 95.2% and the sensitivity was 95.2% at two-digit resolution (92.7 and 95.6%, respectively, at four-digit resolution) for *HLA* alleles with frequency $>2\%$ (Fig. 2B). This indicates that the performance of the imputation was generally excellent for common alleles, consistent with previous work (22). We used *HLA* allele imputations in all participants (even those with *HLA* types defined by sequencing) for association analyses to avoid systematic bias between cases and controls. Lower imputation quality would only decrease power, not increase the false-positive rate, because cases and controls would be equally affected.

We tested all *HLA* alleles for association via logistic regression, adjusting for the same covariates used in SNP analysis (tables S4 and S5). The most significant HLA association is *B*57:01* (OR = 5.5, $P = 1.4 \times 10^{-26}$), which explains the proxy association of rs2395029 in *HCP5*. With the use of stepwise regression modeling in the European sample of controllers and progressors, we were able to implicate *B*57:01*, *B*27:05*, *B*14/Cw*08:02*, *B*52*, and *A*25* as protective alleles and *B*35* and *Cw*07* as risk alleles. These associations are consistent with earlier studies that highlighted a role for HLA class I loci (13–20), and particularly *HLA-B* alleles in control of HIV, which indicated that the imputations are robust. Collectively explaining 19% of the variance of host control, these *HLA* allele associations are consistent with the effects of the four independent SNPs.

Virus-infected cells are recognized by CD8⁺ T cells after presentation of short viral peptides within the binding groove of HLA class I, and HIV-specific CD8⁺ T cells are strongly associated with control (23). We thus evaluated whether the SNP associations identified in the GWAS, and the HLA associations derived from imputation, might be due to specific amino acid positions within the HLA molecules, particularly those involved in the interaction between the viral peptide and the HLA class I molecule. Using the official DNA sequences defined for known *HLA* alleles (24), we encoded all variable amino acid positions within the coding regions of the *HLA* genes in each of the previously *HLA*-typed 2767 individuals in the T1DGC reference panel, and we used this data set to impute the amino acids in the cases and controls (4). Among a total of 372 polymorphic amino acid positions in class I and II HLA proteins, 286 are biallelic like a typical nonsynonymous coding SNP. The remaining 86 positions accommodate more than two amino acids; position 97 is the most diverse in HLA-B with six possible amino acids observed in European populations.

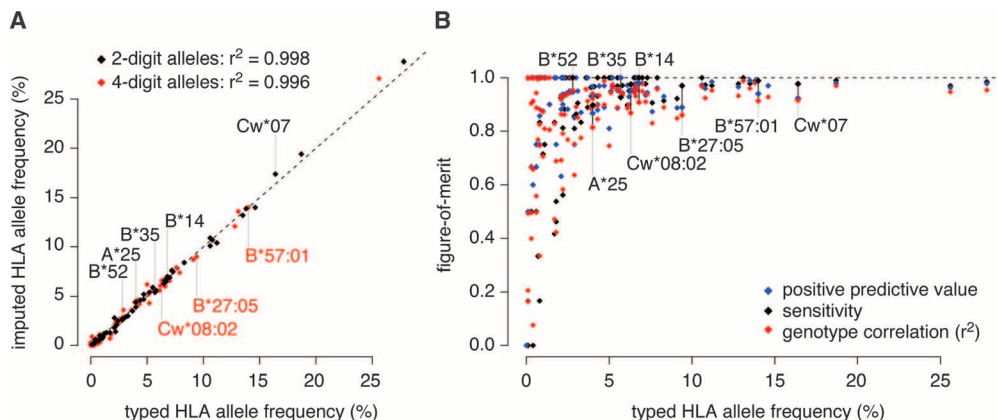
After imputing these amino acids in the European sample, we used logistic regression to test all positions for association with host control (fig. S6 and table S6). Notably, position 97 in HLA-B

was more significant (omnibus $P = 4 \times 10^{-45}$) than any single SNP in the GWAS, and three amino acid positions (67, 70, and 97), all in HLA-B, showed much stronger associations than any single classical *HLA* allele, including *B*57:01* (Fig. 3A). Moreover, allelic variants at these positions were associated with substantial frequency differences between cases and controls (Fig. 3B). These results indicate that the effect of *HLA-B* on disease outcome could be mediated, at least in part, by these positions. These three amino acid positions are located in the peptide binding groove, which suggests that conformational differences in peptide presentation at these sites contribute to the protective or susceptible nature of the various *HLA-B* allotypes. Although both innate and adaptive mechanisms could be at play, the hypothesis that HLA affects peptide presentation and subsequent T cell functionality is supported by experimental data showing substantial functional differences between CTL targeting identical epitopes but restricted by different *HLA* alleles (25).

We next performed stepwise regression modeling and identified six residues as independent markers associated with durable control of HIV. These include Arg⁹⁷, Cys⁶⁷, Gly⁶², and Glu⁶³, all in HLA-B; Ser⁷⁷ in HLA-A; and Met³⁰⁴ in HLA-C, which collectively explain 20% of the observed variance (similar to the variance explained by the seven classical *HLA* alleles described above). With the exception of Met³⁰⁴ in the transmembrane domain of HLA-C, these residues are all located in the MHC class I peptide binding groove, again suggesting that the binding pocket—and, by inference, the conformational presentation of class I-restricted epitopes—plays a key role in host control.

Having identified these amino acid positions as strong candidates to account for the SNP and HLA association signals in this study, we next investigated their effects on protection or risk, revealing allelic variants at these positions linked to both extremes (Table 2). HLA-B position 97 (omnibus $P = 4 \times 10^{-45}$), located at the base of the C pocket, has important conformational properties for peptide binding (26). Position 97 has six allelic variants: Protective haplotypes *B*57:01*, *B*27:05*, and *B*14* are uniquely defined by Val⁹⁷

Fig. 2. Imputation quality of classical *HLA* alleles in the European sample. **(A)** Concordance between imputed (y -axis) and observed (x -axis) frequencies of classical *HLA* types in 371 HIV-1 controllers with four-digit *HLA* types obtained through Sanger sequencing. **(B)** Positive predictive value, sensitivity, and genotype correlation (r^2) with typed alleles as a function of the observed frequency.



(3% frequency in controls), Asn⁹⁷ (4%) and Trp⁹⁷ (3%), respectively; the other amino acids at this position (Ser, Thr, Arg) segregate on a diverse set of haplotypes. Ser⁹⁷ (27% frequency) lies on

risk haplotypes Cw*07, B*07, and others, whereas Thr⁹⁷ (11%) lies on protective B*52 (and others). Arg⁹⁷ is the most common amino acid (51%) and is carried by risk allele B*35, among others.

The importance of this amino acid position to host control is underscored by conditional analyses revealing significance when we adjust incrementally for Val⁹⁷ (omnibus test for position

Fig. 3. Associations at amino acids in *HLA-B* in the European sample. **(A)** Association results for all variable amino acid positions, as calculated by the omnibus test. Colors denote conventional pocket positions. *P* values for significant classical *HLA-B* alleles are shown for comparison. **(B)** Marked allele frequency differences between controllers and progressors for amino acids at positions 67, 70, and 97. Numbers above the bars indicate odds ratios (values >1 indicate a protective effect). **(C)** Associations between allelic variants at amino acid positions 67, 70, and 97 and quantitative virus load set point in the independent Swiss HIV cohort study. Effect estimates (beta coefficients from a linear-regression model) are given in log₁₀ units of virus load set point. *P* values refer to the omnibus test for association at each position. Error bars indicate the standard error of the beta coefficient.

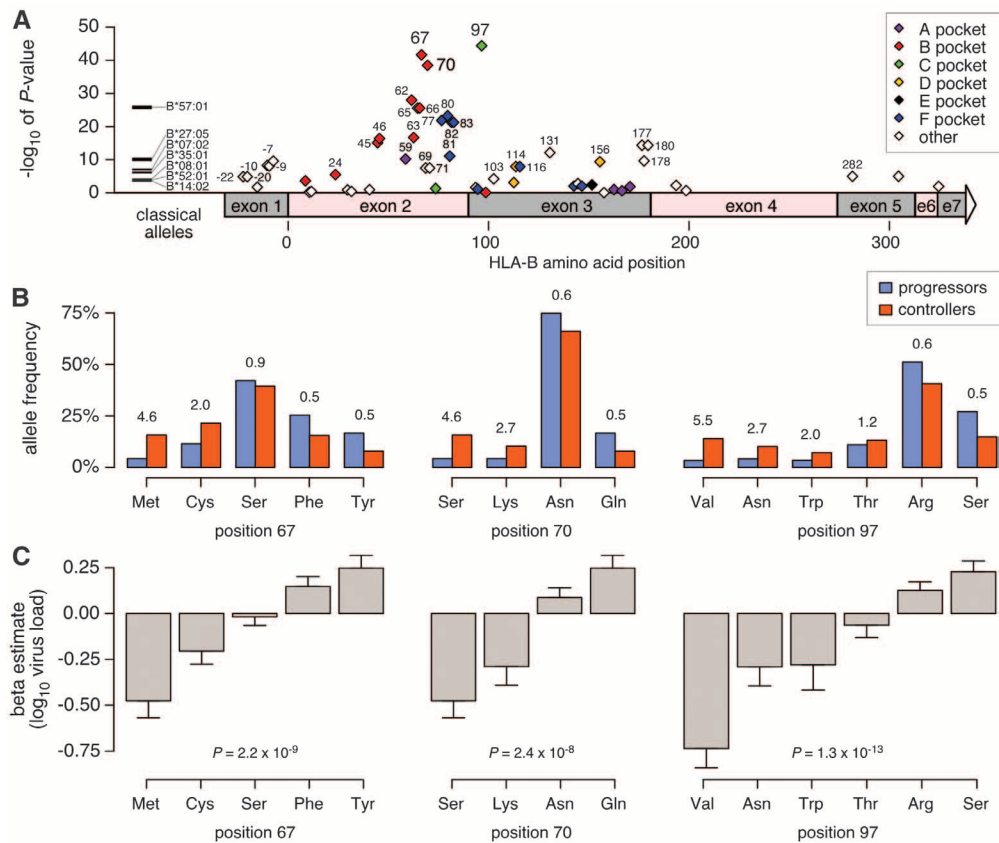


Table 2. Haplotypes defined by the four independent SNPs, classical *HLA* alleles, and amino acids associated with host control in the European sample. Haplotypes are ordered by the estimated odds ratio, where the most common haplotype was taken as reference (OR = 1). *P* values are

for each haplotype tested against all other haplotypes. Only haplotypes with >1% frequency are listed, accounting for >85% of haplotype diversity. *HLA-A* alleles were excluded to limit the number of haplotypes. See (33).

rs3131018	HLA-C		rs9264942	HLA-B						rs4418214	rs2395029	Frequency	OR	P value
	Classical	304		Classical	62	63	67	70	97					
C		M	C	B*57:01	G	E	M	S	V	C	G	0.060	7.05	1.5E-26
C		M	C	B*52:01	R	E	S	N	T	T	T	0.011	6.32	4.2E-05
C		V	C	B*27:05	R	E	C	K	N	C	T	0.051	3.41	1.3E-10
C		M	C		R	E	S	N	T	T	T	0.024	2.78	1.3E-03
C	Cw*08:02	M	C	B*14:02	R	N	C	N	W	T	T	0.030	2.58	6.0E-03
C		M	C		R	N	S	N	R	T	T	0.021	2.16	4.2E-02
C		V	C		R	N	F	N	T	T	T	0.021	2.02	4.6E-01
C		M	C		R	N	C	N	R	T	T	0.025	1.58	1.7E-01
C		V	C		R	E	S	N	S	T	T	0.012	1.50	4.5E-01
A		M	C		R	E	S	N	R	T	T	0.067	1.38	6.5E-01
C		M	T		R	N	F	N	T	T	T	0.020	1.29	8.9E-01
A		M	C		R	N	S	N	R	T	T	0.016	1.03	1.7E-01
C		V	T		R	E	S	N	R	T	T	0.168	(reference)	1.6E-03
C		M	C		R	E	S	N	R	T	T	0.022	0.98	4.4E-01
A		V	T		R	E	S	N	R	T	T	0.018	0.87	6.0E-02
C	Cw*07:01	V	T		R	N	S	N	R	T	T	0.016	0.80	9.5E-02
C	Cw*07:01	V	T	B*08:01	R	N	F	N	S	T	T	0.085	0.79	6.0E-05
C		V	T		R	N	Y	Q	T	T	T	0.018	0.67	3.3E-02
A	Cw*07:02	V	T	B*07:02	R	N	Y	Q	S	T	T	0.116	0.65	3.2E-08
A		V	T	B*35:01	R	N	F	N	R	T	T	0.050	0.51	4.3E-06
A		V	T		R	N	F	N	R	T	T	0.017	0.29	4.1E-05

97, $P = 3 \times 10^{-20}$), Asn⁹⁷ ($P = 2 \times 10^{-9}$), and Trp⁹⁷ ($P = 7 \times 10^{-5}$). Thus, at a single position within the peptide binding groove (position 97, C-pocket), discrete amino acids are associated with opposite disease outcomes, even after controlling for *B*57* and *B*27*, alleles associated with host control.

We also found similar discordant associations for alleles at positions 67, 63, and 62 (Table 2), all of which line the $\alpha 1$ helix along the peptide binding groove and help shape the B-pocket (Fig. 4). At position 67 (omnibus $P = 2 \times 10^{-42}$), risk haplotypes *B*35* and *B*07* carry aromatic residues Phe⁶⁷ and Tyr⁶⁷, respectively, whereas protective *B*57:01*, *B*27:05*, and *B*14* alleles carry sulfur-containing residues Met⁶⁷ or Cys⁶⁷. Position 62 ($P = 5 \times 10^{-27}$) is biallelic (Arg/Gly) with the Gly⁶² allele segregating with protective alleles *B*57:01* and *B*58* (<1% frequency, OR = 1.7, $P = 0.2$). Adjacent position 63 ($P = 9 \times 10^{-16}$) is also biallelic (Glu/Asn) with Glu⁶³ appearing in complete LD ($D' = 1$) with *B*57:01*, *B*27:05*, and *B*52*. In contrast, at this position the risk alleles *B*07* (14% frequency, OR = 0.5, $P = 1 \times 10^{-7}$) and *B*35* both carry Asn⁶³. Position 70 (omnibus $P = 3 \times 10^{-39}$) accommodates four alleles that are tightly coupled with positions 67 and 97: Ser⁷⁰ appears exclusively with Met⁶⁷ (which defines *B*57* and *B*58*), Gln⁷⁰ with Tyr⁶⁷, and Lys⁷⁰ with Asn⁹⁷ (*B*27*). Hence, these data create a consistent and parsimonious model that can explain the associations of classical *HLA-B* alleles by specific amino acids lining the binding groove (and residues tightly coupled to them), which are expected to have an impact on the three-dimensional structure of the peptide-MHC complex.

To further investigate the role of individual amino acid positions in *HLA-B*, we implemented a permutation procedure to assess how consistent the above observations are with a null model in which there is no relation between amino acids at a particular position and host control (4). The

results of this procedure provided evidence that multiple amino acid positions in the peptide binding groove are indeed associated with host control (table S7), including positions 62, 63, 67, 70, and 97, thus providing a structural basis for the effect of *HLA-B* on host control (Fig. 4).

Within *HLA-A* position 77, which lies on the α helix contributing to the F-pocket, we identified a weaker but still significant association (omnibus $P = 3 \times 10^{-6}$). Ser⁷⁷ (6% frequency, OR = 2.0, $P = 2 \times 10^{-6}$) is carried by only two *HLA-A* alleles (joint $r^2 = 1$): *A*25* (2.4% frequency, OR = 2.6, $P = 1 \times 10^{-5}$) and *A*32* (3.2%, OR = 1.6, $P = 0.02$). Given its location and earlier association evidence for the A10 supertype (27), *HLA-A* could play a role in host control, although the evidence is not as strong as for *HLA-B*.

The signals within *HLA-C* are less straightforward to interpret. Position 304 is a biallelic variant (Val/Met) located in the transmembrane domain (Met³⁰⁴, 28% frequency, OR = 2.3, $P = 7 \times 10^{-23}$). Met³⁰⁴ is in moderate LD ($r^2 = 0.5$) with rs9264942, which is known to be associated with *HLA-C* expression levels (28). Addition of this SNP to a multivariate model of all six amino acids is marginally significant ($P = 0.013$) but eliminates the effect of Met³⁰⁴ ($P = 0.06$). Similarly, addition of rs9264942 to a multivariate model of all seven independent classical *HLA* alleles is also significant ($P = 2 \times 10^{-4}$) but eliminates the effect of *Cw*07* ($P = 0.08$). These observations make it difficult to determine the extent to which epitope presentation in the *HLA-C* peptide binding pocket is important for host control. Thus, rs9264942 could be a proxy for not only many protective and risk *HLA* alleles (predominantly at *HLA-B*), but also for an independent effect on *HLA-C* gene expression, differentially affecting the response to HIV (29).

We next evaluated associations for the SNPs in the MHC, classical *HLA* alleles, and amino acids in a second independent cohort of untreated HIV-infected persons from Switzerland (fig. S7 and tables S8 and S9) (4), in whom virus load set point was measured as a quantitative trait. Allelic variants at positions 67, 70, and 97 were also associated with highly significant differences in virus load set point in this second cohort (Fig. 3C). The effect estimates of all variable amino acids in *HLA-B* ($r^2 > 0.9$) and, to a lesser degree, those in *HLA-C* ($r^2 > 0.8$) in that cohort are in excellent agreement (figs. S8 and S9). As before, position 97 in *HLA-B* is the most significant association (omnibus $P = 1 \times 10^{-13}$). The *HLA-A* associations (*A*25* or Ser⁷⁷) did not replicate, which reduces the likelihood that *HLA-A* plays a major role in host control.

In the African American sample (fig. S10), the most significant *HLA* allele association was observed for two-digit *B*57* (OR = 5.1, $P = 1.7 \times 10^{-21}$) and four-digit *B*57:03* (OR = 5.1, $P = 2.8 \times 10^{-17}$; tables S10 and S11), consistent with previous studies (11–13). Position 97 in *HLA-B* (omnibus $P = 2 \times 10^{-25}$) is again the most significant amino acid (table S12). The consistency

of these results demonstrates that imputation and association testing at amino acid resolution in multiple ethnicities can resolve disparate SNP associations in the MHC and help with fine-mapping of classical *HLA* associations.

Altogether, these results link the major genetic impact of host control of HIV-1 to specific amino acids involved in the presentation of viral peptides on infected cells. Moreover, they reconcile previously reported SNP and *HLA* associations with host control and lack of control to specific amino acid positions within the MHC class I peptide binding groove. Although variation in the entire *HLA* protein is involved in the differential response to HIV across *HLA* allotypes, the major genetic effects are condensed to the positions highlighted in this study, indicating a structural basis for the *HLA* association with disease progression that is probably mediated by the conformation of the peptide within the class I binding groove. The most significant residue, position 97 in the floor of the peptide binding groove of *HLA-B*, is associated with the extremes of viral load, depending on the expressed amino acid. This residue has been shown to have important conformational properties that affect epitope-contacting residues within the binding groove (26, 30) and has also been implicated in *HLA* protein folding and cell-surface expression (31).

Although the main focus of this study was on common sequence variation, it remains an open question as to the role of variants outside the MHC and the contribution of epistatic effects and epigenetic regulation. Additional factors also contribute to immune control of HIV, including fitness-altering mutations, immunoregulatory networks, T cell help, thymic selection, and innate effector mechanisms such as killer cell immunoglobulin-like receptor recognition (23), some of which are influenced by the peptide-*HLA* class I complex. However, the combination and location of the significant amino acids defined here are most consistent with the genetic associations observed being modulated by *HLA* class I restricted CD8⁺ T cells. These results implicate the nature of the *HLA*-viral peptide interaction as the major genetic factor modulating durable control of HIV infection and provide the basis for future studies of the impact of *HLA*-peptide conformation on immune cell induction and function.

References and Notes

1. A. J. McMichael, P. Borrow, G. D. Tomaras, N. Goonetilleke, B. F. Haynes, *Nat. Rev. Immunol.* **10**, 11 (2010).
2. S. G. Deeks, B. D. Walker, *Immunity* **27**, 406 (2007).
3. The International HapMap 3 Consortium, *Nature* **461**, 52 (2010).
4. See supporting online material on Science Online for detailed background on the analyses that we performed.
5. M. Dean et al., *Science* **273**, 1856 (1996).
6. M. P. Martin et al., *Science* **282**, 1907 (1998).
7. M. W. Smith et al., *Science* **277**, 959 (1997).
8. P. I. W. de Bakker et al., *Nat. Genet.* **38**, 1166 (2006).
9. J. Fellay et al., *Science* **317**, 944 (2007); 10.1126/science.1143767.

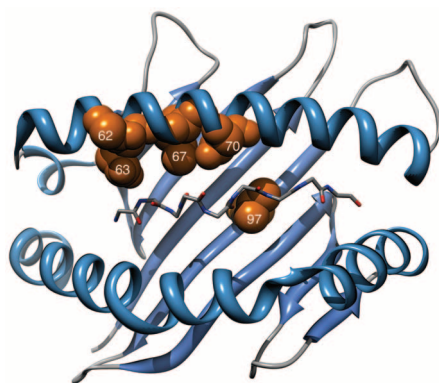


Fig. 4. Three-dimensional ribbon representation of the *HLA-B* protein based on Protein Data Bank entry 2bvp (30), highlighting amino acid positions 62, 63, 67, 70, and 97 lining the peptide binding pocket. The peptide backbone of the epitope is also displayed. This figure was prepared with UCSF Chimera (32).

10. N. J. D. Nagelkerke, *Biometrika* **78**, 691 (1991).
11. K. Pelak *et al.*, *J. Infect. Dis.* **201**, 1141 (2010).
12. F. Pereyra *et al.*, *J. Infect. Dis.* **197**, 563 (2008).
13. C. Costello *et al.*, *AIDS* **13**, 1990 (1999).
14. M. R. Klein *et al.*, *J. Infect. Dis.* **169**, 1244 (1994).
15. R. A. Kaslow *et al.*, *Nat. Med.* **2**, 405 (1996).
16. M. Carrington *et al.*, *Science* **283**, 1748 (1999).
17. S. A. Migueles *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 2709 (2000).
18. P. O. Flores-Villanueva *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 5140 (2001).
19. M. Carrington, S. J. O'Brien, *Annu. Rev. Med.* **54**, 535 (2003).
20. P. Kiepiela *et al.*, *Nature* **432**, 769 (2004).
21. W. M. Brown *et al.*, *Diabetes Obes. Metab.* **11** (suppl. 1), 2 (2009).
22. S. Leslie, P. Donnelly, G. McVean, *Am. J. Hum. Genet.* **82**, 48 (2008).
23. H. W. Virgin, B. D. Walker, *Nature* **464**, 224 (2010).
24. J. Robinson *et al.*, *Nucleic Acids Res.* **37**, D1013 (2009).
25. A. Leslie *et al.*, *J. Immunol.* **177**, 4699 (2006).
26. T. Fagerberg, J. C. Cerottini, O. Michielin, *J. Mol. Biol.* **356**, 521 (2006).
27. G. Catano *et al.*, *PLoS ONE* **3**, e3636 (2008).
28. B. E. Stranger *et al.*, *Nat. Genet.* **39**, 1217 (2007).
29. R. Thomas *et al.*, *Nat. Genet.* **41**, 1290 (2009).
30. G. B. Stewart-Jones *et al.*, *J. Immunol.* **175**, 2459 (2005).
31. M. A. Blanco-Gelaz *et al.*, *Int. Immunol.* **18**, 211 (2006).
32. E. F. Pettersen *et al.*, *J. Comput. Chem.* **25**, 1605 (2004).
33. Single-letter abbreviations for the amino acid residues referred to in Table 2 are as follows: E, Glu; F, Phe; G, Gly; K, Lys; M, Met; N, Asn; Q, Gln; R, Arg; S, Ser; V, Val; W, Trp; and Y, Tyr.
34. This work was made possible through a generous donation from the Mark and Lisa Schwartz Foundation and a subsequent award from the Collaboration for AIDS Vaccine Discovery of the Bill and Melinda Gates Foundation. This work was also supported in part by the Harvard University Center for AIDS Research (grant P-30-AI060354); University of California San Francisco (UCSF) Center for AIDS Research (grant P-30-AI27763); UCSF Clinical and Translational Science Institute (grant UL1 RR024131); Center for AIDS Research Network of Integrated Clinical Systems (grant R24 AI067039); and NIH grants AI28568 and AI030914 (B.D.W.); AI087145 and K24AI069994 (S.G.D.); AI069513, AI34835, AI069432, AI069423, AI069477, AI069501, AI069474, AI069428, AI69467, AI069415, AI32782, AI27661, AI25859, AI28568, AI030914, AI069495, AI069471, AI069532, AI069452, AI069450, AI069556, AI069484, AI069472, AI34853, AI069465, AI069511, AI38844, AI069424, AI069434, AI46370, AI68634, AI069502, AI069419, AI068636, and R024975 (AIDS Clinical Trials Group); and AI077505 and MH071205 (D.W.H.). The Swiss HIV Cohort Study is supported by the Swiss National Science Foundation (SNF grants 33CSO-108787 and 310000-110012). S. Ripke acknowledges support from NIH/National Institute of Mental Health (grant MH085520). This project has been funded in whole or in part with funds from National Cancer Institute/NIH (grant HHSN261200800001E to M. Carrington). The content of this publication does not necessarily reflect the views or policies of the U.S. Department of Health and Human Services, nor does the mention of trade names, commercial products, or organizations imply endorsement by the U.S. government. This research was supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

Author Contributions

Writing team: Florencia Pereyra,^{1,2,†} Xiaoming Jia,^{3,†} Paul J. McLaren,^{4,5,†} Amalio Telenti,⁶ Paul I.W. de Bakker,^{4,5,7,8,†} (co-chair), Bruce D. Walker,^{1,9,†} (co-chair)
Analysis team: Xiaoming Jia,³ Paul J. McLaren,^{4,5} (project leaders), Stephan Ripke,^{4,10} Chanson J. Brumme,¹ Sara L. Pulit,^{4,5} Amalio Telenti,⁶ Mary Carrington,^{1,11} Carl M. Kadie,¹² Jonathan M.

Carlson,¹³ David Heckerman,¹³ Paul I.W. de Bakker,^{4,5,7,8,†} (chair)
Study design: Florencia Pereyra,^{1,2} Paul I.W. de Bakker,^{4,5,7,8,†} Robert R. Graham,^{1,4} Robert M. Plenge,^{4,15} Steven G. Deeks,¹⁶ Bruce D. Walker,^{1,9,†} (chair)
SNP genotyping, HLA typing, and sample management: Lauren Gianniny,⁴ Gabriel Crawford,⁴ Jordan Sullivan,⁴ Elena Gonzalez,⁴ Leela Davies,⁴ Amy Camargo,⁴ Jamie M. Moore,⁴ Nicole Beattie,⁴ Supriya Gupta,⁴ Andrew Crenshaw,⁴ Noël P. Burt,⁴ Candace Guiducci,⁴ Namrata Gupta,⁴ Mary Carrington,^{1,11} Xiaojiang Gao,¹¹ Ying Qi,¹¹ Yuku Yuki¹¹
HIV controllers recruitment and sample management: Florencia Pereyra^{1,2} (project leader), Alicia Piechocka-Trocha,¹ Emily Cutrell,¹ Rachel Rosenberg,¹ Kristin L. Moss,¹ Paul Lemay,¹ Jessica O'Leary,¹ Todd Schaefer,¹ Pranshu Verma,¹ Ildiko Toth,¹ Brian Block,¹ Brett Baker,¹ Alissa Rothchild,¹ Jeffrey Lian,¹ Jacqueline Proudfoot,¹ Donna Marie L. Alvino,¹ Seanna Vine,¹ Marylyn M. Addo,¹ Todd M. Allen,¹ Marcus Altfeld,¹ Matthew R. Henn,⁴ Sylvie Le Gall,¹ Hendrik Streeck,¹ Bruce D. Walker^{1,9,†} (chair)
AIDS Clinical Trials Group: David W. Haas,¹⁷ Daniel R. Kuritzkes,² Gregory K. Robbins,¹⁸ Robert W. Shafer,¹⁹ Roy M. Gulick,²⁰ Cecilia M. Shikuma,²¹ Richard Haubrich,²² Sharon Riddler,²³ Paul E. Sax,²⁴ Eric S. Daar,²⁴ Heather J. Ribaud,²⁵ **HIV controllers referral team:** Brian Agan,²⁶ Shanu Agarwal,²⁷ Richard L. Aherne,¹⁸ Brady L. Allen,²⁸ Sherly Altidor,²⁹ Eric L. Altschuler,³⁰ Sujata Ambardar,³¹ Kathryn Anastos,³² Ben Anderson,³³ Val Anderson,³⁴ Ushan Andrady,³⁴ Diana Antoniskis,³⁵ David Bangsberg,^{1,18} Daniel Barbaro,³⁶ William Barrie,³⁷ J. Bartczak,³⁸ Simon Barton,³⁹ Patricia Basden,⁴⁰ Nesli Basgoz,¹⁸ Suzanne Bazner,⁴¹ Nicholas C. Bellos,⁴¹ Anne M. Benson,⁴⁰ Judith Berger,⁴² Nicole F. Bernard,⁴³ Annette M. Bernard,⁴⁴ Christopher Birch,¹ Stanley J. Bodner,⁴⁵ Robert K. Bolan,⁴⁶ Emilie T. Boudreaux,⁴⁷ Meg Bradley,¹ James F. Braun,⁴⁸ Jon E. Brndjar,⁴⁹ Stephen J. Brown,⁵⁰ Katherine Brown,⁵¹ Sheldon T. Brown,⁵² Jediah Burack,⁵³ Larry M. Bush,⁵⁴ Virginia Cafaro,⁵⁵ Omololaj Campbell,¹⁸ John Campbell,⁵⁶ Robert H. Carlson,⁵⁷ J. Kevin Carmichael,⁵⁸ Kathleen K. Casey,⁵⁹ Chris Cavacuiti,⁶⁰ Gregory Celestin,⁶¹ Steven T. Chambers,⁶² Nancy Chez,⁶³ Lisa M. Church,⁶⁴ Paul J. Cimoche,⁶⁵ Daniel Cohen,⁶⁶ Lillian E. Cohn,⁶⁷ Brian Conaway,⁶⁸ David A. Cooper,⁶⁹ Brian Cornelison,⁶⁰ David T. Cox,⁷⁰ Michael V. Cristofano,⁷¹ George Cuchural Jr.,⁷² Julie L. Czartoski,⁷³ Joseph M. Dahman,⁷⁴ Jennifer S. Daly,⁷⁵ Benjamin T. Davis,¹⁸ Kristine Davis,⁷⁶ Sheila M. Davod,¹⁸ Steven G. Deeks,¹⁶ Edwin DeJesus,⁷⁷ Craig A. Dietz,⁷⁸ Eleanor Dunham,⁶⁴ Michael E. Dunn,⁷⁹ Todd B. Ellerlin,⁸⁰ Joseph J. Eron,⁸¹ John J.W. Fangman,⁸² Claire E. Farel,⁸² Helen Ferlazzo,⁸³ Anita Fidler,⁸⁴ Anita Fleener-Ford,⁸⁵ Renee Frankel,⁸⁶ Kenneth A. Freedberg,¹⁸ Neel K. French,⁸⁷ Jonathan D. Fuchs,⁸⁸ Jon D. Fuller,⁸⁹ Jonna Gaberman,⁹⁰ Joel E. Gallant,⁹¹ Rajesh T. Gandhi,¹⁸ Efrain Garcia,⁹² Donald Garmon,⁹³ Joseph C. Gathe Jr.,⁹⁴ Cyril R. Gaultier,⁹⁵ Wondwoosen Gebre,⁹⁶ Frank D. Gilman,⁹⁷ Ian Gilson,⁹⁸ Paul A. Goepfert,⁹⁹ Michael S. Gottlieb,¹⁰⁰ Claudia Goulston,¹⁰¹ Richard K. Groger,¹⁰² T. Douglas Gurley,¹⁰³ Stuart Haber,¹⁰⁴ Robin Hardwick,¹⁰⁵ W. David Hardy,²⁴ P. Richard Harrigan,¹⁰⁶ Trevor N. Hawkins,¹⁰⁷ Sonya Heath,⁹⁹ Frederick M. Hecht,¹⁶ W. Keith Henry,¹⁰⁸ Melissa Hladek,¹⁰⁹ Robert P. Hoffman,¹¹⁰ James M. Horton,¹¹¹ Ricky K. Hsu,¹¹² Gregory D. Huhn,¹¹³ Peter Hunt,¹⁶ Mark J. Hupert,³⁶ Mark L. Illeman,¹¹⁴ Hans Jaeger,¹¹⁵ Robert M. Jellinger,¹¹⁶ Mina John,¹¹⁷ Jennifer A. Johnson,¹¹⁸ Robert L. Johnson,¹⁸ Heather Johnson,³⁶ Kay Johnson,¹¹⁸ Jennifer Joly,⁶⁴ Wilbert C. Jordan,¹¹⁹ Carol A. Kauffman,¹²⁰ Homayoun Khanlou,¹²¹ Robert K. Killian,¹²² Arthur Y. Kim,¹²³ David D. Kim,¹²³ Clifford A. Kinder,¹²⁴ Jeffrey T. Kirchner,¹²⁵ Laura Kogelman,¹²⁶ Ema Milunka Kojic,¹²⁷ P. Todd Korthuis,¹²⁸ Wayne Kurisu,⁹⁷ Douglas S. Kwon,¹ Melissa LaMar,⁹⁷ Harry Lampiris,¹⁶ Massimiliano Lanzafame,¹²⁹ Michael M. Lederman,¹³⁰ David M. Lee,²⁸ Jean M.L. Lee,⁷³ Marah J. Lee,¹³¹ Edward T.Y. Lee,¹³² Janice Lemoine,¹³³ Jay A. Levy,¹⁶ Josep M. Llibre,¹³⁴ Michael A. Liguori,¹¹² Susan J. Little,²² Anne Y. Liu,² Alvaro J. Lopez,¹³⁵ Mono R. Loutfy,¹³⁶ Dawn Loy,¹³⁷ Debbie Y. Mohammed,³⁰ Alan Man,³⁵ Michael K. Mansour,¹⁸ Vincent C. Marconi,¹³⁸ Martin Markowitz,¹³⁹ Rui Marques,¹⁴⁰ Jeffrey N. Martin,¹⁶ Harold L. Martin Jr.,¹⁴¹ Kenneth Hugh Mayer,⁶⁶ M. Juliana McElrath,⁷³ Theresa A. McGhee,¹⁴² Barbara H. McGovern,¹²⁶ Katherine McGowan,² Dawn McIntyre,⁵⁹ Gavin X. McLeod,¹⁴³ Prema Menezes,⁶¹ Greg Mesa,¹⁴⁴ Craig E. Metroka,²⁹ Dirk Meyer-Olson,¹⁴⁵ Andy O. Miller,¹⁴⁶ Kate Montgomery,¹⁴⁷ Karam C. Mounzer,¹⁴⁸ Ellen H. Nagami,¹ Iris Nagin,¹⁴⁹ Ronald G. Nahass,¹⁵⁰ Margaret O. Nelson,¹⁸ Craig

Nielsen,¹⁵¹ David L. Norene,¹⁵² David H. O'Connor,¹⁵³ Bisola O. Ojikutu,¹⁸ Jason Okulicz,¹⁵⁴ Olakunle O. Oladehin,¹⁸ Edward C. Oldfield III,¹⁵⁵ Susan A. Olender,¹⁵⁶ Mario Ostrowski,¹⁵⁶ William F. Owen Jr.,¹⁵⁷ Eunice Pae,¹ Jeffrey Parsonnet,¹⁵⁸ Andrew M. Pavlatos,¹⁵⁹ Aaron M. Perlmuter,¹⁶⁰ Michael N. Pierce,²¹⁸ Jonathan M. Pincus,¹⁶¹ Leandro Pisani,¹⁶² Lawrence Jay Price,¹⁶³ Laurie Proia,¹⁶⁴ Richard C. Prokesch,¹³⁷ Heather Calderon Pujet,¹⁶⁵ Moti Ramgopal,¹⁶⁶ Almas Rathod,¹ Michael Rausch,¹⁶⁷ J. Ravishankar,¹⁶⁸ Frank S. Rhame,¹⁶⁹ Constance Shamyarira Richards,¹⁷⁰ Douglas D. Richman,²² Gregory K. Robbins,¹⁸ Berta Rodes,¹⁷¹ Milagros Rodriguez,¹⁶² Richard C. Ross II,¹⁷² Eric S. Rosenberg,¹⁸ Daniel Rosenthal,¹⁷³ Polly E. Ross,¹⁷⁴ David S. Rubin,¹⁷⁵ Elase Rumbaugh,³⁵ Luis Saenz,¹⁶² Michelle R. Salvaggio,¹⁷⁶ William C. Sanchez,¹⁷⁷ Veerav M. Sanjana,¹⁷⁸ Steven Santiago,¹⁶² Wolfgang Schmidt,¹⁷⁹ Hanneke Schuitemaker,¹⁸⁰ Philip M. Sestak,¹⁸¹ Peter Shalit,¹⁸² William Shay,¹⁰⁴ Vivian N. Shirvani,¹⁸³ Vanessa I. Silebi,¹⁸⁴ James M. Sizemore Jr.,¹⁸⁵ Paul R. Skolnik,⁸⁹ Marcia Sokol-Anderson,¹⁸⁶ James M. Sosman,¹⁵³ Paul Stabile,¹⁸⁷ Jack T. Stapleton,¹⁸⁸ Sheree Starrett,¹⁸⁹ Francine Stein,⁸³ Hans-Jürgen Stellbrink,¹⁹⁰ F. Lisa Sterman,¹⁹¹ Valerie E. Stone,¹⁸ David R. Stone,¹⁹² Giuseppe Tambussi,¹⁹³ Randy A. Taplitz,²² Ellen M. Tedaldi,¹⁹⁴ Amalio Telenti,⁶ William Theisen,² Richard Torres,¹⁹⁵ Lorraine Tosiello,¹⁹⁶ Cecile Tremblay,¹⁹⁷ Marc A. Tribble,¹⁹⁸ Phuong D. Trinh,¹⁹⁹ Alice Tsao,¹ Peggy Ueda,¹ Anthony Vaccaro,²⁰⁰ Emilia Valadas,²⁰¹ Thanes J. Vanig,²⁰² Isabel Vecino,²⁰³ Vilma M. Vega,¹³⁷ Wenoah Veikley,¹⁰⁷ Barbara H. Wade,²⁰⁴ Charles Walworth,⁶⁵ Chingchai Wanidworanun,²⁰⁵ Douglas J. Ward,²⁰⁶ Daniel A. Warner,²⁰⁷ Robert D. Weber,²⁰⁸ Duncan Webster,²⁰⁹ Steve Weis,²⁰³ David A. Wheeler,²¹⁰ David J. White,²¹¹ Ed Wilkins,²¹² Alan Winston,⁸⁴ Clifford G. Wlodawer,²¹³ Angelique van't Wout,¹⁸⁰ David P. Wright,²¹⁴ Otto O. Yang,²⁴ David L. Yuridin,²¹⁵ Brandon W. Zabukovic,²¹⁶ Kimon C. Zachary,¹⁸ Beth Zeeman,¹ Meng Zhao²¹⁷

¹Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology (MIT) and Harvard, Boston, MA, USA. ²Department of Medicine, Division of Infectious Disease, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. ³Harvard-MIT Division of Health Sciences and Technology, Boston, MA, USA. ⁴Broad Institute of Harvard and MIT, Cambridge, MA, USA. ⁵Department of Medicine, Division of Genetics, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. ⁶Institute of Microbiology, University of Lausanne, Lausanne, Switzerland. ⁷Department of Medical Genetics, Division of Biomedical Genetics, University Medical Center Utrecht, Netherlands. ⁸Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Netherlands. ⁹Howard Hughes Medical Institute, Chevy Chase, MD, USA. ¹⁰Department of Medicine, Center for Human Genetic Research, MGH, Harvard Medical School, Boston, MA, USA. ¹¹Cancer and Inflammation Program, Laboratory of Experimental Immunology, SAIC-Frederick, NCI-Frederick, Frederick, MD, USA. ¹²Microsoft Research, Redmond, WA, USA. ¹³Microsoft Research, Los Angeles, CA, USA. ¹⁴Genentech, South San Francisco, CA, USA. ¹⁵Department of Medicine, Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. ¹⁶University of California San Francisco, San Francisco, CA, USA. ¹⁷Vanderbilt University School of Medicine, Nashville, TN, USA. ¹⁸MGH, Harvard Medical School, Boston, MA, USA. ¹⁹Stanford University, Palo Alto, CA, USA. ²⁰Weill Medical College of Cornell University, New York, NY, USA. ²¹Hawaii Center for AIDS, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI, USA. ²²University of California San Diego, San Diego, CA, USA. ²³University of Pittsburgh, Pittsburgh, PA, USA. ²⁴University of California Los Angeles, Los Angeles, CA, USA. ²⁵Department of Biostatistics, Harvard School of Public Health, Boston, MA, USA. ²⁶Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences, Bethesda, MD, USA. ²⁷Summa Health System, Akron, OH, USA. ²⁸Uptown Physicians Group, Dallas, TX, USA. ²⁹St. Luke's Roosevelt Hospital, New York, NY, USA. ³⁰New Jersey Medical School, University Hospital, Newark, NJ, USA. ³¹Infectious Disease Physicians, Annandale, VA, USA. ³²Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY, USA. ³³St. Leonards Medical Centre, St. Leonards, Australia. ³⁴Syby Gwynedd Hospital, Gwynedd, UK. ³⁵Kaiser Permanente, Portland, OR, USA. ³⁶Tarrant County Infectious Disease Associates, Fort Worth, TX, USA. ³⁷Private Practice of William Barrie,

- Toronto, Canada. ³⁸Rowan Tree Medical, Wilton Manors, FL, USA. ³⁹Chelsea and Westminster Hospital, St. Stephen's Centre, London, UK. ⁴⁰Beaver Street Family Practice, Flagstaff, AZ, USA. ⁴¹Southwest Infectious Disease Associates, Dallas, TX, USA. ⁴²St. Barnabas Hospital, Bronx, NY, USA. ⁴³Research Institute, McGill University Health Centre, Montreal General Hospital, Montreal, Canada. ⁴⁴Thacker, Thompson and Bernard, Atlanta, GA, USA. ⁴⁵Vanderbilt University School of Medicine, Hermitage, TN, USA. ⁴⁶LA Gay and Lesbian Center, Los Angeles, CA, USA. ⁴⁷Louisiana State University Health Sciences Center, University Medical Center East Clinic, Lafayette, LA, USA. ⁴⁸Physicians' Research Network, Callen-Lorde Community Health Center, New York, NY, USA. ⁴⁹Brndjar Medical Associates, Allentown, PA, USA. ⁵⁰AIDS Research Alliance, Los Angeles, CA, USA. ⁵¹David Powell Community Health Center, Austin, TX, USA. ⁵²James J. Peters VA Medical Center, Bronx, NY, USA. ⁵³Sunrise Medical Group, Brooklyn, NY, USA. ⁵⁴University of Miami-Miller School of Medicine, Lake Worth, FL, USA. ⁵⁵WellSpring Medical Group, San Francisco, CA, USA. ⁵⁶Moses Cone Health System, Greensboro, NC, USA. ⁵⁷HealthPartners Infectious Disease, St Paul, MN, USA. ⁵⁸El Rio Special Immunology Associates, Tucson, AZ, USA. ⁵⁹Jersey Shore University Medical Center, Neptune, NJ, USA. ⁶⁰St. Michaels Hospital, Toronto, Canada. ⁶¹The Brooklyn Hospital Center, PATH Center, Brooklyn, NY, USA. ⁶²University of Otago, Christchurch, New Zealand. ⁶³H.E.L.P./Project Samaritan, Bronx, NY, USA. ⁶⁴David E. Rogers Center for HIV/AIDS Care, Southampton, NY, USA. ⁶⁵Center for Special Immunology, Fountain Valley, CA, USA. ⁶⁶Fenway Community Health, Boston, MA, USA. ⁶⁷9th Street Internal Medicine Associates, Philadelphia, PA, USA. ⁶⁸University of British Columbia, Vancouver, Canada. ⁶⁹National Centre in HIV Epidemiology and Clinical Research, Sydney, Australia. ⁷⁰Metro Infectious Disease Consultants, Indianapolis, IN, USA. ⁷¹John H. Stroger Hospital of Cook County, Chicago, IL, USA. ⁷²New England Quality Care Alliance, Braintree, MA, USA. ⁷³Fred Hutchinson Cancer Research Center, Seattle, WA, USA. ⁷⁴Desert AIDS Project, Palm Springs, CA, USA. ⁷⁵University of Massachusetts Memorial Medical Center, Worcester, MA, USA. ⁷⁶University of Iowa Hospitals and Clinics, Iowa City, IA, USA. ⁷⁷Orlando Immunology Center, Orlando, FL, USA. ⁷⁸The Kansas City Free Health Clinic, Kansas City, MO, USA. ⁷⁹Private Practice of Michael E. Dunn, M.D., Tampa, FL, USA. ⁸⁰South Shore Hospital, Weymouth, MA, USA. ⁸¹University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. ⁸²AIDS Resource Center of Wisconsin, Milwaukee, WI, USA. ⁸³Visiting Nurse Association of Central New Jersey, Community Health Center, Asbury Park, NJ, USA. ⁸⁴Imperial College, London, UK. ⁸⁵Heartland Clinic, Paducah, KY, USA. ⁸⁶Morristown Memorial Hospital, Morristown, NJ, USA. ⁸⁷Private Practice of Neel K. French, M.D., Chicago, IL, USA. ⁸⁸San Francisco Department of Public Health, San Francisco, CA, USA. ⁸⁹Boston University Medical Center, Boston, MA, USA. ⁹⁰Baystate Medical Center, Springfield, MA, USA. ⁹¹Johns Hopkins University School of Medicine, Baltimore, MD, USA. ⁹²Private Practice of Efrain Garcia, M.D., Miami, FL, USA. ⁹³The Rockefeller University, New York, NY, USA. ⁹⁴Private Practice of Joseph C. Gathe Jr., M.D., Houston, TX, USA. ⁹⁵Tower Infectious Disease, Los Angeles, CA, USA. ⁹⁶Nassau University Medical Center, East Meadow, NY, USA. ⁹⁷Sharp Rees Stealy Medical Center, San Diego, CA, USA. ⁹⁸Medical College of Wisconsin, Milwaukee, WI, USA. ⁹⁹University of Alabama, Birmingham, Birmingham, AL, USA. ¹⁰⁰Synergy Hematology and Oncology, Los Angeles, CA, USA. ¹⁰¹University of Utah, Salt Lake City, UT, USA. ¹⁰²South Dayton Acute Care Consultants, Dayton, OH, USA. ¹⁰³T. Douglas Gurley, M.D., Atlanta, GA, USA. ¹⁰⁴St. Vincent's Hospital, New York, NY, USA. ¹⁰⁵University of Texas Health Science Center, Houston, TX, USA. ¹⁰⁶British Columbia Centre for Excellence in HIV/AIDS, Vancouver, Canada. ¹⁰⁷Southwest C.A.R.E. Center, Santa Fe, NM, USA. ¹⁰⁸Hennepin County Medical Center, Minneapolis, MN, USA. ¹⁰⁹The Catholic University of America, School of Nursing, Washington, DC, USA. ¹¹⁰Mercy Medical Center, Springfield, MA, USA. ¹¹¹CMC Myers Park Medical Center, Charlotte, NC, USA. ¹¹²New York University Medical Center, New York, NY, USA. ¹¹³The Ruth M. Rothshon Care Center, Chicago, IL, USA. ¹¹⁴Feldman Medical Group, San Francisco, CA, USA. ¹¹⁵HIV Research and Clinical Care Centre, Munich, Germany. ¹¹⁶Albany Medical College, Albany, NY, USA. ¹¹⁷Murdoch University, Murdoch, Australia. ¹¹⁸University of Cincinnati, Cincinnati, OH, USA. ¹¹⁹OASIS Clinic, Los Angeles, CA, USA. ¹²⁰VA Ann Arbor Healthcare System, Ann Arbor, MI, USA. ¹²¹AIDS Healthcare Foundation, Los Angeles, CA, USA. ¹²²Capitol Hill Medical, Seattle, WA, USA. ¹²³Astor Medical Group, New York, NY, USA. ¹²⁴The Kinder Medical Group, Miami, FL, USA. ¹²⁵Lancaster General Hospital, Lancaster, PA, USA. ¹²⁶Tufts Medical Center, Boston, MA, USA. ¹²⁷Alpert Medical School of Brown University, Providence, RI, USA. ¹²⁸Oregon Health and Science University, Portland, OR, USA. ¹²⁹G.B. Rossi Hospital, Verona, Italy. ¹³⁰Case Western Reserve University, Cleveland, OH, USA. ¹³¹LifeWay, Fort Lauderdale, FL, USA. ¹³²Saint Claire Medical Associate, Toronto, Canada. ¹³³Greater Lawrence Family Health Center, Lawrence, MA, USA. ¹³⁴Hospital Universitari Germans Trias i Pujol, Barcelona, Spain. ¹³⁵Infectious Disease Consultants, Tucker, GA, USA. ¹³⁶University of Toronto, Toronto, Canada. ¹³⁷Infectious Disease Associates, Sarasota, FL, USA. ¹³⁸Emory University, School of Medicine, Atlanta, GA, USA. ¹³⁹Aaron Diamond AIDS Research Center, Rockefeller University, New York, NY, USA. ¹⁴⁰Deruico Doencas Infecciosas, Porto, Portugal. ¹⁴¹Park Nicollet Clinic, St. Louis, MN, USA. ¹⁴²Absolute Care, Atlanta, GA, USA. ¹⁴³College of Physicians and Surgeons, Columbia University, New York, NY, USA. ¹⁴⁴Highland Medical Associates, Hendersonville, NC, USA. ¹⁴⁵Medizinische Hochschule, Abteilung Klinische Immunologie, Hannover, Germany. ¹⁴⁶Hospital for Special Surgery, New York, NY, USA. ¹⁴⁷Family Practice Specialists, Phoenix, AZ, USA. ¹⁴⁸Philadelphia FIGHT, Philadelphia, PA, USA. ¹⁴⁹Lower East Side Service Center, New York, NY, USA. ¹⁵⁰Infectious Diseases Care, Hillsborough, NJ, USA. ¹⁵¹University of Colorado, Denver, Aurora, CO, USA. ¹⁵²Sutter Medical Group, Sacramento, CA, USA. ¹⁵³University of Wisconsin in Madison, Madison, WI, USA. ¹⁵⁴Brooke Army Medical Center, San Antonio, TX, USA. ¹⁵⁵Eastern Virginia Medical School, Norfolk, VA, USA. ¹⁵⁶Columbia University Medical Center, New York, NY, USA. ¹⁵⁷CA Pacific Medical Center, San Francisco, CA, USA. ¹⁵⁸Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA. ¹⁵⁹St. Joseph Hospital, Chicago, IL, USA. ¹⁶⁰Aaron M. Perlmuter, M.D., Beverly Hills, CA, USA. ¹⁶¹Codman Square Health Center, Dorchester, MA, USA. ¹⁶²CARE Resource, Miami, FL, USA. ¹⁶³Castro-Mission Health Center, San Francisco, CA, USA. ¹⁶⁴Rush Medical College, Chicago, IL, USA. ¹⁶⁵Boulder Community Hospital, Boulder, CO, USA. ¹⁶⁶Midway Immunology and Research Center, Fort Pierce, FL, USA. ¹⁶⁷Aerztezentrum Nollendorfplatz, Berlin, Germany. ¹⁶⁸State University of New York Downstate Medical Center, Brooklyn, NY, USA. ¹⁶⁹Clinic 42, Minneapolis, MN, USA. ¹⁷⁰King Edward Memorial Hospital, Paget, Bermuda. ¹⁷¹Fundacion para la Investigacion Biomedica del Hospital Carlos III, Madrid, Spain. ¹⁷²Summit Medical Group, Knoxville, TN, USA. ¹⁷³Medical Consultants of South Florida, Coral Springs, FL, USA. ¹⁷⁴Western North Carolina Community Health Services, Asheville, NC, USA. ¹⁷⁵New York Hospital Medical Center of Queens, Flushing, NY, USA. ¹⁷⁶University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA. ¹⁷⁷Georgetown University Medical Center, Washington, DC, USA. ¹⁷⁸Village Care Health Center, New York, NY, USA. ¹⁷⁹Aerzteforum Seestrasse, Berlin, Germany. ¹⁸⁰Academic Medical Center Amsterdam, Amsterdam, Netherlands. ¹⁸¹St. Paul's Hospital, Vancouver, Canada. ¹⁸²Swedish Medical Center, Seattle, WA, USA. ¹⁸³Cedars-Sinai Medical Center, Los Angeles, CA, USA. ¹⁸⁴Mercy Hospital, Miami, FL, USA. ¹⁸⁵University of Tennessee, Chattanooga, TN, USA. ¹⁸⁶St. Louis University, St. Louis, MO, USA. ¹⁸⁷William F. Ryan Community Health Center, New York, NY, USA. ¹⁸⁸The University of Iowa, Iowa City, IA, USA. ¹⁸⁹Rivington House and Village Care, New York, NY, USA. ¹⁹⁰Infektionsmedizinisches Centrum Hamburg, Hamburg, Germany. ¹⁹¹California Pacific Medical Center, San Francisco, CA, USA. ¹⁹²Lemuel Shattuck Hospital, Boston, MA, USA. ¹⁹³Fondazione San Raffaele Del Monte Tabor, Milan, Italy. ¹⁹⁴Temple University School of Medicine, Philadelphia, PA, USA. ¹⁹⁵Yale University School of Medicine, Bridgeport, CT, USA. ¹⁹⁶Jersey City Medical Center, Jersey City, NJ, USA. ¹⁹⁷University of Montreal, Montreal, Canada. ¹⁹⁸Baylor University Medical Center, Dallas, TX, USA. ¹⁹⁹Montgomery Infectious Disease Associates, Silver Spring, MD, USA. ²⁰⁰Northwestern University, Chicago, IL, USA. ²⁰¹Hospital de Santa Maria, Faculdade de Medicina de Lisboa, Lisbon, Portugal. ²⁰²Spectrum Medical Group, Phoenix, AZ, USA. ²⁰³University of North Texas Health Science Center, Fort Worth, TX, USA. ²⁰⁴Infectious Diseases Associates of Northwest Florida, Pensacola, FL, USA. ²⁰⁵Private Practice of Chingchai Wanidworanun, M.D., Arlington, VA, USA. ²⁰⁶Dupont Circle Physician's Group, Washington, DC, USA. ²⁰⁷Consultative Medicine, Daytona Beach, FL, USA. ²⁰⁸Infectious Disease Specialists, Colorado Springs, CO, USA. ²⁰⁹Saint John Regional Hospital, Saint John, Canada. ²¹⁰Clinical Alliance for Research and Education-Infectious Diseases, Annandale, VA, USA. ²¹¹Hawthorn House, Birmingham Heartlands Hospital, Birmingham, UK. ²¹²North Manchester General Hospital, Manchester, UK. ²¹³Private Practice of Clifford Wlodaver, Midwest City, OK, USA. ²¹⁴Central Texas Clinical Research, Austin, TX, USA. ²¹⁵Primary Health Care, Des Moines, IA, USA. ²¹⁶Memorial Neighborhood Health Center Central Clinic, South Bend, IN, USA. ²¹⁷United Health Services Hospitals, Binghamton, NY, USA. ²¹⁸All Med and Rehabilitation of New York, Bronx, NY, USA.

†These authors contributed equally to this work.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1195271/DC1
Materials and Methods

Figs. S1 to S12

Tables S1 to S13

References

19 July 2010; accepted 26 October 2010

Published online 4 November 2010;

10.1126/science.1195271



Science Careers Classified Advertising

For full advertising details, go to ScienceCareers.org and click For Employers, or call one of our representatives.

Tracy Holmes

Worldwide Associate Director
Science Careers
Phone: +44 (0) 1223 326525

UNITED STATES & CANADA

E-mail: advertise@sciencecareers.org
Fax: 202-289-6742

Tina Burks

Midwest/West Coast/
South Central/Canada
Phone: 202-326-6577

Elizabeth Early

East Coast & Industry
Phone: 202-326-6578

Marci Gallun

Sales Administrator
Phone: 202-326-6582

Online Job Posting Questions

Phone: 202-326-6577

EUROPE & REST OF WORLD

E-mail: ads@science-int.co.uk
Fax: +44 (0) 1223 326532

Alex Palmer

Phone: +44 (0) 1223 326527

Susanne Kharraz Tavakol

Phone: +44 (0) 1223 326529

Dan Pennington

Phone: +44 (0) 1223 326517

Lisa Patterson

Phone: +44 (0) 1223 326528

JAPAN

ASCA Corporation

Jie Chin
Phone: +81-3-6802-4616
Fax: +81-3-6802-4615
E-mail: careerads@sciencemag.jp

To subscribe to Science:

In United States call 866-434-2227
In the rest of the world call +1 202-326-6417

All ads submitted for publication must comply with applicable U.S. and non-U.S. laws. *Science* reserves the right to refuse any advertisement at its sole discretion for any reason, including without limitation for offensive language or inappropriate content, and all advertising is subject to publisher approval. *Science* encourages our readers to alert us to any ads that they feel may be discriminatory or offensive.

Science Careers

From the journal *Science* 

POSITIONS OPEN



POSTDOCTORAL FELLOW, PH.D., AND M.SC. POSITIONS NSERC Canadian Aquatic Invasive Species Network II

The Canadian Aquatic Invasive Species Network II, a global leader in the study of aquatic invasive species, has one Postdoctoral, eight Ph.D., and four M.Sc. positions open in research institutions across Canada. Working in conjunction with the network's partners, primarily shipping companies and government agencies, your research will assist in the development of innovative early detection technology and rapid response capabilities that will help preserve marine and freshwater habitats and along Canada's four coastlines.

Please visit our CAISN II Job Board at [website: http://www.caisn.ca](http://www.caisn.ca) to discover more information about specific positions, locations, and contact information.

FACULTY POSITION IN GROSS ANATOMY

The Department of Anatomy, Physiology and Pharmacology, within the College of Veterinary Medicine at Auburn University, invites applications for a full-time, 12-month tenure-track position in Gross Anatomy at the ASSISTANT/ASSOCIATE PROFESSOR level. Minimum qualifications include a D.V.M. degree (or equivalent) and/or Ph.D. in Biomedical Sciences or an allied field. Responsibilities will include team-teaching of gross anatomy in the veterinary curriculum. Preference will be given to candidates with prior experience teaching the subject of Gross Anatomy. The successful candidate will also be expected to develop an externally funded research program. The candidate selected for this position must be able to meet eligibility requirements to work in the United States at the time the appointment is scheduled to begin, and continue working legally for the proposed term of employment. Excellent communication skills are required. Applicants should submit: (1) letter describing teaching and research experience and career goals, (2) curriculum vitae, and (3) names, mailing addresses, telephone numbers, and e-mail addresses of three references to: Ms. Hattie Alvis, Department of Anatomy, Physiology and Pharmacology, 109 Greene Hall, College of Veterinary Medicine, Auburn University, AL 36849. Formal inquiries can be directed to Dr. Benson Akingbemi, Chairman, Search Committee: e-mail: akingbr@auburn.edu; telephone: 334-844-4489. Interested persons can also visit our website: <http://www.vetmed.auburn.edu/index.pl/employment>. Review of applications will begin January 31, 2011, and continue until a candidate is recommended for hire. Auburn University is an Affirmative Action/Equal Opportunity Employer. Women and Minorities are encouraged to apply.

RNA BIOLOGIST—Florida International University (FIU). The Department of Biological Sciences at FIU invites applications for a nine month, open rank, tenure-track faculty position. We seek applications from highly qualified individuals investigating small non-coding RNAs, with specific emphasis on the role of non-coding RNA in the response to cellular stress and disease. The successful applicant will be expected to develop an extramurally funded research program, and commit to teaching excellence at both the undergraduate and graduate levels. A Ph.D. or equivalent degree in molecular biology or biochemistry is required. Applicants applying at the rank of Assistant Professor should have postdoctoral research and teaching experience, and a strong publication record. Applicants for Associate or Full Professor must have a demonstrated history of research productivity and a strong record of external funding. Please send curriculum vitae, statements of research and teaching philosophy, and contact information for three references to: Kenneth Murray, Department of Biological Sciences, Florida International University, Miami, FL 33199. Electronic applications are encouraged and should be sent to e-mail: kmurray@fiu.edu. Florida International University is an Equal Opportunity Educator and Employer.

POSITIONS OPEN

FACULTY POSITION Microbial Pathogenesis

Applications are invited for faculty positions in microbial pathogenesis at the Public Health Research Institute, an infectious diseases research center at New Jersey Medical School-UMDNJ in Newark, New Jersey. Applicants with an interest in emerging infectious disease pathogens and bio-defense with expertise in host-pathogen interactions are particularly encouraged to apply. The successful candidate will have demonstrated research productivity with existing funding, and he/she will be expected to maintain an independent, innovative NIH-funded research program. A competitive startup package and outstanding core facilities are available, including animal imaging, informatics, and extensive BSL-3/ABSL-3 containment facilities, including a new Regional Biocontainment Laboratory.

Applicants should submit curriculum vitae, a statement of research experience, a summary of future plans, and names of three references by December 15, 2010, to: Dr. Issar Smith, e-mail: smithis@umdnj.edu. Website: <http://www.phri.org>.

Affirmative Action/Equal Employment Opportunity Employer, Minorities/Females/Persons with Disabilities/Veterans.

ASSISTANT PROFESSOR MICROBIOLOGIST

The Biological Sciences Department at SUNY Cortland seeks applications for a tenure-track Assistant Professor starting August 2011. The successful applicant will teach majors courses (Microbiology and possibly a course in their area of specialization), Microbiology and Human Disease for Health Science majors, and participate in Principles of Biology for non-majors. The candidate is expected to seek external funding to maintain a productive research program that engages undergraduates, particularly those interested in the biomedical sciences. Requirements include a Ph.D. in microbiology or related field, and demonstration of effective teaching ability and scholarly productivity. The Biological Sciences Department includes 14 tenured/tenure-track faculty committed to excellence in teaching, scholarly productivity, and the involvement of undergraduates in research. SUNY Cortland is a comprehensive liberal arts college located in close proximity to major research institutions in Ithaca, Binghamton, and Syracuse, New York. For application instructions and to apply go to website: <http://jobs.cortland.edu/applicants/Central?quickFind=52477>. Preference will be given to applications received by January 21, 2011.

SUNY Cortland is an Affirmative Action/Equal Employment Opportunity/ADA Employer. We have a strong commitment to the affirmation of diversity and have interdisciplinary degree programs in the areas of Multicultural Studies.

A POSTDOCTORAL ASSOCIATE position is available for a talented, energetic individual interested in neural regulation of cardiovascular responses to exercise in health and disease within the Lillehei Heart Institute (LHI) at the University of Minnesota Twin Cities Campus. A solid background in whole animal neurophysiology is preferred. In these studies, we will study mechanisms that mediate abnormalities in the exercise pressor reflex in heart failure. The laboratory is supported by NIH, private foundation grants, and intramural funding and is well equipped with modern tools for physiological and molecular studies. Research is supported by established core laboratories within the LHI for confocal imaging, microarray, echocardiographic, facs, and physiological and metabolic testing. Recent Doctoral graduates or individuals in the early years of postdoctoral study should send curriculum vitae and the names of three references electronically to e-mail: garry002@umn.edu. The University of Minnesota is an Affirmative Action/Equal Opportunity Employer.

Max-Planck-Institut für
Eisenforschung GmbH
Düsseldorf (Germany)



We announce an opening for the position of a

Max Planck Director

The Max-Planck-Institut (www.mpie.de) is devoted to fundamental research on complex materials with a strong focus on metals, composites, nanostructured alloys, semiconductors, and functional surfaces. Applications of interest are in the fields of mobility, health, energy, synthesis, and alloy design. We are looking for candidates with an interdisciplinary research profile in

Materials Science and Engineering

The successful candidate will be appointed as head of a department at the full professor level (W3) and Scientific Member of the Max Planck Society. We provide a competitive research environment at highest international level with strong facets in theory, experiment, characterization, and synthesis. We are interested in a colleague willing to strongly cooperate with the existing departments. Experts in the field at a junior and senior research level and with strong scientific record are encouraged to apply.

We are an equal-opportunity employer. Applications should be sent before 31. January 2011 via post or email (as pdf) to:

Max-Planck-Institut für Eisenforschung GmbH
Geschäftsführung
Max-Planck-Str. 1 • 40237 Düsseldorf
director-eifo@mpie.de

SECTION CHIEFS, PRINCIPAL INVESTIGATORS & TECHNICAL DIRECTORS

Shanghai Center for Comprehensive Protein Science (SCPS)

Chinese Academy of Sciences (CAS)

Shanghai Institute of Biochemistry and Cell Biology (SIBCB)

Shanghai Institutes for Biological Sciences (SIBS)

Chinese Academy of Sciences

中国科学院上海蛋白质科学综合研究中心
上海生物化学与细胞生物学研究所
中国科学院上海生命科学研究院

Shanghai Institute of Biochemistry and Cell Biology (SIBCB), SIBS, CAS invites applications for the positions of Section Chiefs, Principal Investigators, and Technical Directors of Shanghai Center for Comprehensive Protein Science (SCPS), CAS. SIBCB, a member institute of SIBS, currently has 54 research groups and over 350 scientists working on diverse aspects of molecular and cellular biology, such as gene regulation, epigenetics, protein sciences, signal transduction, cell and stem cell biology, cancer, and other disease models. SCPS will be housed in the National Protein Science Facility (NPSF)-Shanghai, which is the largest protein science platform in China and is being constructed and managed by SIBCB and SIBS. SCPS will conduct research and provide service in many key areas of protein science, and will be composed of six sections: X-ray Crystallography, Nuclear Magnetic Resonance & Electron Microscopy, Proteomics, Animal Imaging, Bioinformatics, and Animal Facility.

The successful candidate for the Section Chief position must be a tenured associate or full professor in protein science. The section chief will be responsible for administrating the overall research and service activities of the section as well as maintaining an active research program.

The successful candidate for the Principal Investigator position will have a Ph.D. and postdoctoral experience in appropriate fields. The principal investigator will develop an extramurally funded research program and supervise Ph.D. students.

The successful candidate for the Technical Director position will have a Ph.D. in appropriate fields (postdoctoral experience preferred). The technical director will be responsible for the running and maintenance of facilities in SCPS.

Interested applicants should send his/her curriculum vitae, a list of publications, a brief summary of previous accomplishment and future research/work plan, and three letters of references to: Mrs. Jinfang Song, SCPS Faculty Search Committee, SIBCB, SIBS, CAS, 320 Yue-Yang Road, Shanghai 200031, China, or electronically to e-mail: songjif@sibs.ac.cn (telephone: 086-21-54921022 and fax: 086-21-54921011). SIBCB is an Equal Opportunity Employer. For more information, please visit websites: <http://www.sibcb.ac.cn> or <http://www.sibs.ac.cn>

**FRED HUTCHINSON
CANCER RESEARCH CENTER**

A LIFE OF SCIENCE

Human Biology Division Director

The Division of Human Biology at the Fred Hutchinson Cancer Research Center (FHCRC) is soliciting applications for a dynamic and visionary Division Director who can lead the expansion of the solid tumor research program within the Division and across the Center. FHCRC, in partnership with the University of Washington and Children's Hospital and Regional Medical Center, is an established NCI-designated comprehensive cancer center with vibrant research programs that include studies of cancer pathogenesis, prevention, early detection, treatment, and outcomes. An important area for development involves the integration of basic, clinical, and population sciences with translational studies of solid tumor biology.

The ideal candidate will have demonstrated excellence and leadership in translational research emphasizing cancer biology that spans the interface between laboratory, clinical, and/or population-based studies. All aspects of cancer-related research are of interest, from those utilizing large-scale genetic and genomic approaches to those focused on a mechanistic understanding of cancer pathogenesis. The candidate must be able to lead the expansion of the Human Biology Division through additional faculty recruitment, emphasizing translational research in solid tumors, while also fostering the broader research portfolio of the Division.

The Human Biology Division encompasses a diversity of interdisciplinary, collaborative research at the interfaces of basic, clinical, and population sciences in order to further our understanding of human biology, cancer, and other complex human disorders. The Division occupies state-of-the-art research laboratories on a beautiful lakeside campus. The Center offers outstanding shared resources, including genomics, proteomics, imaging, and animal models. The Center has active training programs for graduate students, postdoctoral fellows and clinically oriented trainees, and offers exceptional opportunities for scientific interactions with other investigators in the Seattle area.

Additional information about the Division and Center can be found at: <http://www.fhcrc.org/science/humanbio/> and <http://www.fhcrc.org/science/>

Candidates should submit a curriculum vitae, research and leadership statements, and contact information for three references to:

Human Biology Division Director Search
Fred Hutchinson Cancer Research Center
Mailstop: C3-168
1100 Fairview Avenue North
P.O. Box 19024
Seattle, WA 98109-1024
or:
dljackso@fhcrc.org

Applications will be considered as received until **February 15, 2011** or the position is filled.

The Fred Hutchinson Cancer Research Center is an Equal Opportunity Employer committed to work force diversity. Applications from female and minority candidates are strongly encouraged.



Faculty Positions

FIU is a multi-campus public research university located in Miami, a vibrant, international city. FIU offers more than 180 baccalaureate, masters, professional, and doctoral degree programs to over 42,000 students. As one of South Florida's anchor institutions, FIU is worlds ahead in its local and global engagement and is committed to finding solutions to the most challenging problems of our times.

Herbert Wertheim College of Medicine at Florida International University is seeking to expand its teaching faculty to provide for increasing enrollment.

The HWCOC is accepting applications for 6 full time faculty positions in Basic Medical Sciences and one position in Clinical Pharmacology. The College is seeking individuals committed to guiding student learning who have demonstrated excellence in teaching the basic sciences in an interdisciplinary format in an allopathic medical curriculum. Faculty are expected to teach basic sciences in a longitudinal, three year period of progressive study integrating basic and clinical medicine and to work collaboratively with clinical faculty in the curriculum. Individuals with ability to participate in a multidisciplinary format are preferred. Areas of primary interest in order of preference are: physiology, pharmacology, neuroscience, medical genetics, microbiology, cellular biology and immunology. Preference may be given to individuals with outstanding teaching credentials who are able to teach effectively in more than a single discipline. The primary expectations for the faculty are for major teaching and curriculum leadership roles. Research experience and conducting research independently or in collaboration with HWCOC faculty is encouraged and an option for these positions.

Rank is open and dependent on experience and qualifications. Minimum qualifications include a terminal degree in the medical sciences (PhD, MD, DVM, and PharmD for the Clinical Pharmacology position) experience teaching in an allopathic medical curriculum and student or peer evaluations of teaching quality. The positions will be filled in a rolling manner and will remain open until all are filled. Applications will be accepted immediately and reviewed as received. Positions are available for appointment between October, 2010 and March 2011 and faculty will be expected to be in residence full time by May 2011.

For more information or to apply, please visit us on-line at <http://www.fiujobs.org> and reference position number 42297/ 42298/ 42355/ 42356/ 42357/ 42358. Qualified applicants must submit a letter of interest accompanying their curriculum vitae with names and addresses of three professional references. The CV may include allopathic medical school teaching experience and references may include identification of individuals qualified to evaluate teaching effectiveness. Teaching experience should be clearly stated in the CV including references for peer reviewed published work in medical education. Do not submit teaching materials, course syllabi, course schedules, etc. with an application.



FIU is a member of the State University System of Florida and is an Equal Opportunity, Equal Access Affirmative Action Employer.



Aalto University

Aalto University is a new university created from the merger of the Helsinki School of Economics, the University of Art and Design Helsinki, and the Helsinki University of Technology.

Aalto University invites applications for:

Tenure track or tenured position in Computational science (complex systems)

The position is located in the Department of Biomedical Engineering and Computational Science (BECS) (<http://www.becs.tkk.fi>) of Aalto University, School of Science.

The position is open to talented individuals who hold a doctorate and have excellent potential for a productive scientific career. On the basis of their experience and competence, applicants will be placed on any of the four levels of the tenure track system: Assistant Professor (1), Assistant Professor (2), Associate Professor or Full Professor. The candidates for the two first-mentioned levels are appointed for a fixed term, while the candidates for Associate Professor positions are appointed either for an indefinite term or for a fixed term. A Full Professor is appointed for an indefinite term.

The holder of the professorship is expected to perform outstanding research, to teach at graduate and undergraduate levels, to supervise graduate and undergraduate students, as well as to participate in international collaborations and to raise research funds. The focus areas for the research and teaching of the position are those of the Centre of Excellence in Computational Complex Systems Research (COSY) (<http://www.lce.hut.fi/>), namely Computational Science including modeling large-scale networks, e.g., social, techno-social, economic, ecological, epidemiological, cognitive and neural networks. The main emphasis will be on complex systems research of large-scale, system-wide phenomena and dynamics using and developing the tools of computational science and statistical mechanics.

To apply, please send your CV, a concise description of research interest & future research plans, a short teaching portfolio and contact information of at least 2 persons, who can give recommendations, addressed to the President of Aalto University, Registry of Aalto University, P.O.Box 11000, FI-00076 Aalto, or preferably by email: kirjaamo@aalto.fi. The closing date is 14th of January 2011. Should there be a lack of eligible outstanding applicants, the application period may be extended. While all the applicants who have submitted an application by the deadline will be appropriately considered, Aalto University reserves the right to consider also other candidates for the professorial positions.

The application materials will not be returned.

More detailed information about the BECS and the COSY and their research fields, as well as the present application process and its requirements, is provided online at <http://www.becs.tkk.fi/en/work/tenuretrack2010.html> and <http://www.aalto.fi/en/openpositions>.

www.aalto.fi



Max Planck Society

Max Planck Society seeks faculty in Autonomous Systems at all levels

The Max Planck Society is establishing a major new research direction in "Autonomous Systems" to investigate and understand the organizing principles of autonomous systems that successfully interact with complex environments, and to use this understanding to design future systems. The Institute will study principles of perception, action and learning in material, biological, bio-hybrid, and computational systems ranging from nano to macro scales.

Applications from outstanding candidates at all levels (W2 and W3) are solicited. Senior candidates must have demonstrated leadership abilities and recognized international stature. Applications in PDF format should include a current CV, a research statement relating to autonomous systems, and the names and contact information of 3-5 references. All materials should be sent to apply@as.mpg.de by January 31, 2011.

The Institute will have two sites, Stuttgart and Tübingen, to foster collaboration with existing MPIS, local universities, and centers of excellence. Faculty receive generous funding, unparalleled facilities, and internationally competitive compensation packages. Applications from outstanding researchers are sought regardless of national origin or citizenship; knowledge of the German language is not required.

For more information, see <http://as.mpg.de>

The Max Planck Society is committed to increasing the representation of minorities, women and individuals with physical disabilities in the sciences. We particularly encourage such individuals to apply.

The Max Planck Society is funded by the German federal government and the federal states. Within the Max Planck Society, the regulations that apply with respect to W2 and W3 positions are comparable to federal civil service law.

Successful candidates should be prepared to participate in a symposium on April 11 and 12, 2011.

POST-DOCTORAL RESEARCHER



Penn Medicine

The Smell and Taste Center of the University of Pennsylvania is seeking a PhD-level researcher with expertise in human electrophysiology (EEG). Functional imaging experience ideal but not essential. The position involves conducting, analyzing, and designing experiments, as well as participating in an active smell and taste disorders clinic. Good statistical skills required. A goal of the program is to determine the earliest sensory changes (visual, auditory, vestibular, tactile, gustatory, olfactory) that occur in neurodegenerative diseases and how such changes may be used in early diagnosis. This position provides a unique opportunity to interact with well-established investigators in a number of disciplines at one of the premier universities of the United States. The University of Pennsylvania is an affirmative action/equal opportunity employer strongly committed to gender and ethnic diversity. Ph.D., M.D. or other postdoctoral degree required.

Interested individuals should send cover letter, CV, and reference information, via e-mail, to: Richard L. Doty, Ph.D., University of Pennsylvania, Department of Otorhinolaryngology-Head and Neck Surgery, Division of Smell & Taste, 3400 Spruce Street, Philadelphia, PA 19104. Email: richard.doty@uphs.upenn.edu



The Nanoscience Cooperative Research Center **CIC nanoGUNE Consolider** invites applications and nominations for three tenured positions as **Group Leaders**

The anticipated hires are part of an ongoing expansion strategy to broaden the scope of nanoGUNE's research portfolio as well as complement the already ongoing scientific activities.

CIC nanoGUNE Consolider, located in San Sebastian, Basque Country (Spain), is a recently created R&D center with the mission of conducting basic and applied world-class research in nanoscience and nanotechnology, fostering training and education excellence, and supporting the growth of a nanotechnology based industry. In January of 2009, a new 6.200 m² facility was inaugurated as part of an overall 40 M€ strategic investment. Details about nanoGUNE and the presently conducted research work can be found on our website: www.nanogune.eu

Group Leaders at nanoGUNE are responsible for the design and operation of their respective Research Laboratories. At the present time, nanoGUNE is welcoming applicants with interest in one of the following areas:

- Probe Microscopy and related techniques (#001)
- Materials Synthesis or Nano-scale Chemistry (#002)
- Nano-biotechnology or Nano-medicine (#003)

Candidates should have an outstanding track record of research with an orientation towards nanoscience and nanotechnology, a proven ability to obtain competitive research funding, and a record of technology-transfer initiatives. Proficiency in spoken and written English is compulsory; knowledge of Spanish is not a requirement.

Applicants should forward their CV, a summary of research interests, and a list of at least three references to director@nanogune.eu

Closing date: **9 January 2011**

COLUMBIA UNIVERSITY Department of Biological Sciences and Department of Physics

The Department of Biological Sciences and the Department of Physics of Columbia University invite applications for a joint position in biophysics. (We anticipate that the appointment will be at the Assistant Professor level, but we will also consider senior applicants who could fill the position as Associate Professor—tenured or nontenured—or full Professor with tenure.) Our departments have a long history of leadership in modern biology and physics with an interdisciplinary focus. We are looking for individuals with outstanding and innovative research records and ambitious future plans, bridging the biological and physical sciences. We define biophysics (or biological physics) very broadly, including for example, the development of new techniques and methods, imaging/microscopy, structural and single molecule studies, molecular dynamics, biopolymers, nanobiology, membranes, cellular biomechanics, neuronal circuits, and theoretical approaches. Laboratory space will be available on the main campus of Columbia University, in a new science building (<http://evpr.columbia.edu/content/northwest-corner-building>) devoted to interdisciplinary science, and surrounded by research groups from different departments and schools. We expect that the successful candidate will develop a vigorous research program, provide synergy to the existing research on our campus and participate in undergraduate and graduate teaching.

Minimum Qualifications: Ph.D. Successful predoctoral work as well as successful postdoctoral record, except in exceptional cases where predoctoral record identifies extraordinary candidate.

Review of applications begins immediately and will continue until the position is filled. To be considered as an applicant, you must follow the instructions and apply at the link below:

<http://academicjobs.columbia.edu/applicants/Central?quickFind=54044>

Columbia University is an equal opportunity/affirmative action employer.

Applications/Expressions of Interest Invited for Key Positions with Qatar Biobank

Applications are sought for positions in a major new biomedical research programme conducted by the School of Public Health, Imperial College London in collaboration with the Qatar Foundation for Education, Science and Community Development, and Qatar's Supreme Council of Health. The Qatar Biobank will screen and collect data and biological samples from 100,000+ participants, forming an internationally competitive platform for research into the genetic and environmental determinants of the major diseases. The Biobank will be established and run by a team of research, clinical operations and management staff based in Doha, Qatar, with other key executive, technical and research posts based at Imperial College London.

We currently seek formal applications for the following positions:

Chief Operating Officer, London (SM200-10a) Director of Clinical Operations, Doha (SM200-10c) Chronic Disease Epidemiologist, Doha (SM200-10e)
Chief Technology Officer, London (SM200-10b) Programme Manager, Doha (SM200-10d) Nutritional Epidemiologist, Doha (SM200-10f)

Competitive salary packages to be offered according to qualifications and experience.

Our preferred method of application is online via our website <http://www3.imperial.ac.uk/employment>. Please complete and upload an application form as directed by 5pm GMT Friday 14th January 2011.

Additionally, expressions of interest and/or CVs are sought for the following positions:

Project Manager, London Statistician, London Laboratory Operations Manager, Doha
Chronic Disease Epidemiologist, London ICT Specialist, London Data Analyst, London
Clinical Co-ordinator, Doha

Other management, academic and technical posts also available.

For further details on the School of Public Health, see our website: www.imperial.ac.uk/medicine/about/divisions/publichealth

For informal discussions or to express interest in the general positions, please contact the Project Directors, Professor Paul Elliott and Professor Elio Riboli, by emailing qatarbiobank@imperial.ac.uk. Please state your area of interest and expertise when contacting us.

Committed to equality and valuing diversity. We are also an Athena Silver SWAN Award winner, a Stonewall Diversity Champion and a Stonewall Top 100 Employer 2010.



CORPORATE POSTDOCTORAL RESIDENCY PROGRAM

Life Technologies and Keck Graduate Institute of Applied Life Sciences invite outstanding researchers with a PhD degree in science or technology to apply for the Corporate Postdoctoral Residency Program. This unique program leads to both corporate experience and the innovative Postdoctoral Professional Masters (PPM) degree in Bioscience Management from Keck Graduate Institute.

The program sequence consists of:

- A summer internship in the laboratory of a senior investigator at Life Technologies.
- Fall enrollment at Keck Graduate Institute in the PPM; a 9-month accredited masters program that helps PhDs acquire the business and management skills needed to pursue either R&D or management careers within the life sciences industry.
- Upon completion of the PPM degree, the postdoctoral residents return to Life Technologies to participate in a 1-year residency program.

This 24-month corporate experience and networking opportunity is unsurpassed for postdoctoral researchers interested in opportunities in the life sciences industry. The Corporate Postdoctoral Residency Program has been designed by the Chief Scientific Officer and the Global Head of R&D of Life Technologies along with KGI's academic leadership. It provides a unique opportunity for Postdoctoral scientists and engineers to gain experience in the corporate life sciences community, while maintaining and utilizing their unique scientific expertise.

Please apply by **February 1, 2010** to be considered for this program. For further information about the program and our PPM visitation day, see www.kgi.edu or e-mail admissions@kgi.edu.

Open tenure track positions

The Faculty of Chemistry and Materials Sciences at Aalto University conducts academic research in the fields of Biotechnology and Chemical Technology, Chemistry and Forest Products Technology and Materials Science and Engineering. The research results and innovations made in the faculty are widely applied in the society and industry.

The faculty invites applications for 4 tenure track positions:

Biobased Material Technology with the primary focus on the production of materials that are composed at least partially of wood and/or other plant fibers and/or their structural elements.

Bioprocess Engineering with focus on the development of biotechnical production processes as well as new biocatalysts and microorganisms.

Bioproduct Chemistry with the primary focus on the chemistry of wood and other plant polymers and substances and their uses in materials and other applications.

Industrial Chemistry with focus on catalysis and chemical reaction technology. Research may also include thermodynamics, chemical kinetics, reactor design and control of product quality.

Successful candidates are expected to have a D.Sc./Ph.D. in a relevant scientific discipline, a track record of excellence in research and potential to develop an outstanding research program.

The candidates are expected to engage in teaching and development of research programs together with faculty and students. The positions are located in Espoo, Finland. The complete position descriptions with more information on preferred qualifications are available at www.aalto.fi/en/current/jobs/.

Aalto University is a new university building on the three most prestigious higher education institutions in Finland, including Helsinki University of Technology. The new university's ambitious goal is to rank among the top universities in the world in its areas of specialization.



Faculty Positions in a Multidisciplinary Initiative in Pharmacology, Chemical Biology and Metabolomics

A campus-wide initiative at Washington University School of Medicine and the Saint Louis College of Pharmacy aims to hire 6 or more faculty members working in the areas of basic pharmacology, chemical biology, pharmacogenomics, metabolomics, and drug disposition. These faculty will have key roles in a growing community of pharmacological scientists. The quantitative analysis of cellular metabolites plays an increasingly important role in understanding responses to disease and drug therapies. Correspondingly, small molecule probes that modulate physiological responses in health and rectify maladaptive alterations during disease will lead to the development of novel treatments.

The Departments of Biochemistry and Molecular Biophysics, Genetics, Anesthesiology, Medicine, Pathology & Immunology, Molecular Microbiology, Radiation Oncology, the Siteman Cancer Center and Alzheimer's Disease Research Center at Washington University School of Medicine together with the Saint Louis College of Pharmacy invite applications for tenure-track faculty positions at all academic levels. We are seeking applicants with a strong record of accomplishment in the areas of rational design and synthesis of bioactive small molecules, quantitative analysis of endogenous and drug-derived metabolites, systems-level analyses of metabolic networks, drug target discovery and characterization, genetic influences of variability in drug responses, and clinical evaluation of drug candidates. Enthusiasm for teaching and mentoring young scientists are also important goals of this initiative. Faculty may be appointed in any of the above academic units, or a combination thereof, depending on their qualifications and interests.

Washington University has a highly interactive research environment with vigorous interdisciplinary graduate and medical scientist training programs. St. Louis College of Pharmacy has a 146-year tradition of excellence in educational programs with an expanding mission to support basic research in the pharmacological sciences. Minority and women scientists are especially encouraged to apply. Applicants should submit their curriculum vitae, selected reprints, and a short summary of future research plans to WUpharmsearch@biochem.wustl.edu along with 3 letters of reference. Inquiries should be directed to: (314) 362-0287.

AN EQUAL OPPORTUNITY EMPLOYER.

DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE WEILL CORNELL MEDICAL COLLEGE NEW YORK, NEW YORK

The Department of Pathology and Laboratory Medicine of the Weill Cornell Medical College is seeking candidates for the position of Assistant Professor (tenure-track) in the Center for Vascular Biology, Department of Pathology and Laboratory Medicine. Individuals with demonstrated accomplishments in the following areas are encouraged to apply - angiogenesis, tumor microenvironment, vascular cell and molecular biology, mechanisms of inflammation, systems biology of the vascular system, gene expression mechanisms and RNA-based signaling mechanisms. Highly competitive research support will be provided in an interactive and nurturing environment. Individuals will have an opportunity to establish a state-of-the art independent research program in newly renovated space and to interact with a strong group of affiliated vascular biologists. Weill Cornell Medical College is part of an allied Tri-Institution on the upper east side of Manhattan, including the Memorial Sloan Kettering Cancer Center and Rockefeller University, and also affiliated with the adjacent New York-Presbyterian Hospital and Hospital for Special Surgery.

Interested individuals should forward their curriculum vitae, complete bibliography, statement of interests, PDFs of three of their best publications, and the names and contact information for three references: **Dr. Timothy Hla, Center for Vascular Biology, Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, 1300 York Avenue, Room A607E/Mailbox 69, New York, NY 10065** or preferably electronically to tih2002@med.cornell.edu. The position is available immediately. Applications will be evaluated upon receipt and will be accepted until the position is filled.

*Weill Cornell Medical College is an
Affirmative Action/Equal Opportunity Employer.*

Assistant Professor, Tenure Track Biochemistry or Molecular Genetics

The Department of Biochemistry and Molecular Biology, University of Louisville, School of Medicine invites applications for a tenure-track position at the Assistant Professor level. The Department is under new leadership and we anticipate that several additional positions will be filled in the next 3 years. Thus, successful candidates will play vital roles in the future development of the Department and the Health Science Center. The Department of Biochemistry and Molecular Biology is part of a vibrant research environment on UofL's Health Sciences Campus, which houses the Schools of Medicine, Dentistry, Nursing, Public Health and Information Sciences. The candidate will be part of vibrant and growing research endeavor that has involved construction of 4 new research buildings, and exceptional core facilities over the last 10 years.

Individuals with expertise in all areas of Biochemistry and Genetics are invited to apply. Those using comparative and genetic approaches with either model organisms, particularly mouse, rat and zebrafish or human studies to understand disease processes are encouraged to apply. Candidates must hold a PhD, MD, DDS or equivalent degree and have a strong potential to maintain a productive research program. As part of an academic unit, successful candidates will also be expected to teach in graduate and either Medical or Dental Biochemistry courses. New faculty will receive a competitive startup package, customized laboratory space, and numerous opportunities for collaborative interactions with departmental and center investigators focusing on cancer, cardiovascular disease, diabetes, obesity, neuroscience, aging, environmental health, and stem cell biology.

Applicants should send a single PDF file containing their research summary, curriculum vitae, and names with contact information of at least three individuals who may be contacted for letters of reference to debbie.powell@louisville.edu or by mail to: **Assistant Professor Search Committee, Department of Biochemistry and Molecular Biology, University of Louisville School of Medicine, Louisville, KY 40292**. Applicants must also apply on-line for **Job ID# 26577** at www.louisville.edu/jobs/. Application review will begin **January 1, 2011** and continue until the position is filled. Applicants must be U.S. citizens, permanent residents, or have a valid H1B1 visa.

The University of Louisville is an Affirmative Action/Equal Opportunity Employer. Women and minorities are strongly encouraged to apply.



Five Center Directorships, multiple vacancies for open-rank tenure-track faculty, and postdoctoral research fellows Frontier Institute of Science and Technology (FIST) Xi'an Jiaotong University (XJTU)

(Valid through May 31, 2011)

FIST is a large selective investment by XJTU in an effort to establish a world-class, multi-disciplinary research institute. To achieve this goal, FIST is setting up 10 research centers of excellence in Physics, Chemistry, Bio-Science/Life-Science/Basic-medical-Science, and Materials Science, and adopts a new management system similar to that of most U.S. universities. Five out of the ten planned centers have been established recently, and FIST is now recruiting the remaining 5 Center Directors (either full-time or honorary). In addition, FIST invites applications to fill its multiple, full-time tenure-track faculty positions at all levels (from lab director to group leader), as well as postdoctoral positions. See our [Chinese ad](#) for details.

An eligible candidate for the Center Director position should be an internationally renowned scientist and established leader in his/her field, with the ability and will to build his/her center into an internationally recognized center of excellence. Successful candidates will be provided with a sizable start-up package to establish a research center, together with a salary (500k-800k RMB for full-time directors) or an honorarium commensurate with the working days (for honorary directors). See our [Chinese ad](#) for details.

In addition to the Center Director positions, FIST also invites applications in the above-mentioned areas to fill its full-time, tenure-track faculty positions at all levels, from lab director to group leader. Applications for postdoctoral positions are also welcome. An eligible faculty candidate should have a track-record for excellence in research and the potential to lead a lab or a group to success. Successful candidates will be provided with a competitive start-up package including a salary of 100k-500k RMB, 15-200m² lab space, and 100k-2 million RMB start-up fund, together with many other benefits. Position level and start-up package will vary with the candidate's qualification. See our [Chinese ad](#) for details.

Interested individuals should set up their free ResearcherID webpage on <http://www.researcherid.com/>. Please send your ResearcherID citation information along with a cover letter, CV, and a list of 10 representative publications to Dr. Xiangli Meng, Frontier Institute of Science and Technology(FIST), Xi'an Jiaotong University, No.1 West building, 99 Yanxiang Road, Yanta District, Xi'an, Shaanxi Province, P.R.China, 710054. Tel/Fax: +86 29 83395131, E-mail: fist@mail.xjtu.edu.cn.

XJTU is an AA/EOE employer.



Indian Institute of Tropical Meteorology (IITM), Pune, India

(An Autonomous Institute of the Ministry of
Earth Sciences, Government of India)

Advertisement No. PER/14/2010/CAT

**Centre for Advanced Training in Earth System
Sciences and Climate (CAT-ESSC)**

**OPPORTUNITIES FOR OUTSTANDING
RESEARCHERS IN CLIMATE SCIENCES**

The newly formed Centre (CAT-ESSC) attached to IITM, an organization fully devoted to research in various aspects of climate sciences, is looking for outstanding researchers to fill **Ten Research cum Teaching Positions** at the level of **Scientists E, D and C. A Doctorate in Meteorology, Atmospheric Sciences, Oceanography, Physics, Mathematics, Applied Mathematics, Statistics, Applied Physics, Geophysics or in any other area of Earth System Sciences or Engineering from a recognized university/ institution and experience of research work preferably related to climate are the essential qualifications.** The selected faculty is expected to conduct cutting edge research on climate related topics and should have a commitment to quality teaching and inclination towards institutional development. They should inspire the trainees of the Centre at the **M.Tech / Ph.D** levels using theoretical, modeling and observational techniques. Aspiring candidates may submit applications, latest by **31 December 2010**. For details of the posts visit http://www.tropmet.res.in/view_jobs.php.



OLD DOMINION UNIVERSITY

MICROBIOLOGY/IMMUNOLOGY
FACULTY POSITIONS

The Department of Biological Sciences, College of Sciences, Old Dominion University, invites applications for several tenure track/tenured Faculty positions at the Assistant Professor, Associate Professor, or Professor level as part of a major recruitment initiative. We are particularly interested in: (1) investigators in host-pathogen interactions and molecular pathogenesis; (2) molecular or cellular immunologists employing contemporary molecular biology approaches. A successful candidate must establish and maintain a vigorous research program that will attract peer-reviewed funding, and provide excellent education to our graduate and undergraduate students. All applicants must have a Ph.D., M.D. or an equivalent degree in an appropriate field. Applicants at the Associate Professor or Professor level must demonstrate substantial research accomplishments, a consistent record of independent peer-reviewed funding, and have active competitive grants. Interactions are encouraged with other departments/units of the College and the University as well as the Eastern Virginia Medical School. A cross-appointment with the Center for Molecular Medicine (Chris D. Platsoucas, Ph.D., Center Director) is available. State salary support and competitive start-up packages are available. The Department of Biological Sciences receives substantial support from state funds as well as from research grants from federal and other granting agencies. The department has strong Ph.D. and M.S. graduate programs that currently enroll over 125 students.

The College of Sciences is undergoing a major research expansion. Over the last three years research grant awards to the College have increased by 78% to \$20.6 million in FY2010. Old Dominion University (www.odu.edu) is a state supported, Carnegie doctoral research extensive institution enrolling more than 24,000 students including 6,000 graduate students.

Interested individuals should submit curriculum vitae, a statement of research achievements and research plans, and the names, addresses, e-mail addresses and phone numbers of three references to **Wayne Hynes, Ph.D., Professor and Chairman, Department of Biological Sciences, Old Dominion University at MICB@odu.edu**. Review of applicants will begin immediately and continue until the positions are filled.

Old Dominion University is an Affirmative Action/Equal Opportunity institution and requires compliance with the Immigration Reform and Control Act of 1986.

MARIAN UNIVERSITY

Indianapolis
A Catholic and Franciscan University

Associate Dean of Biomedical Science

Marian University College of Osteopathic Medicine

Marian University has an opening for an Associate Dean for Biomedical Science

The ideal candidate will have a Ph.D. degree with at least 5 years of executive experience as a medical school department chair or similar position; expertise as a senior academic leader with experience in curricular design, research program participation and supervision, management of budgets and personnel, and demonstrated excellence in teaching. This position reports directly to the Dean of the College of Osteopathic Medicine. Specialty abilities in designing and implementing a new program of instruction, including assessment of learning equipment and technology, are required. The candidate must have knowledge of and commitment to the mission of Marian University; ability to work under pressure in a multi-task environment; exceptional communication skills using verbal, written and electronic mediums to appropriate audiences; broad knowledge of academic and leadership skill sets; ability to successfully complete documentation and requirements from accreditation agencies, and public relations and budget analysis skills.

Located on 114 wooded acres six miles from downtown Indianapolis, Marian University is a Catholic University dedicated to excellent teaching and learning in the Franciscan and liberal arts traditions with degree programs in the arts, sciences, business, education and nursing. Marian has achieved remarkable success in advancing the university in the areas of academic quality, vibrancy of campus life, enhancing the Catholic and Franciscan dimension of the university community, and growth in enrollment and fundraising areas. Additional information on Marian University can be obtained at www.marian.edu.

To apply: Review of applications will begin immediately and continue until the position is filled. Candidates must provide a complete application packet to include a letter of application addressing qualifications for the position, a current curriculum vitae, and the names and addresses of three current references to: **Director of Human Resources, Marian University, 3200 Cold Spring Rd, Indianapolis, IN 46222**. Electronic submission of applications is encouraged to hr@marian.edu.

Only complete applicant packets will be considered. Review of applications will begin immediately and continue until the position is filled.

Marian University is an Equal Opportunity Employer

Assistant or Associate Professors - University of North Dakota School of Medicine and Health Sciences

The School of Medicine and Health Sciences at the University of North Dakota is in the process of expanding its research enterprise with up to 8 faculty positions to be filled in the next few years. Projected research focus areas include neurosciences, infectious disease, cancer and the environment, health disparities, eating disorders, and aging. Three tenure-track faculty positions are available **immediately** in the following fields:

- The Department of Anatomy and Cell Biology seeks an individual whose expertise in **signaling in neurodegenerative disorders** will enhance established and emerging research focus areas in neurodegenerative disorders, including cellular signaling, cellular interactions with the environment, and cell survival. The inter-departmental and cross-disciplinary neuroscience research group is supported in part by substantial COBRE (NIH) funding.
- The Department of Biochemistry and Molecular Biology seeks an individual with expertise in **epigenetics and/or stem cell biology**. The preferred candidate will be able to contribute to the focus area of Cancer and the Environment, but applicants that may contribute to any of the other focus areas above will also be considered. Successful candidates should be open to collaborative opportunities within the department and School.
- The Department of Microbiology and Immunology seeks an **immunologist with expertise in infectious disease**. The preferred candidate will complement current research interests in host immune responses to microbial infections through investigation of the molecular and/or cellular aspects of immunological processes. Scientific expertise in the innate and/or regulatory immunological aspects of host-microbe interactions is preferred.

Applicants for these positions must have a Ph.D., M.D., D.V.M. or equivalent degree in an applicable biomedical science, at least three years of postdoctoral research experience, a history of publishing high quality peer-reviewed manuscripts, and an aptitude and willingness to participate in medical and graduate education. Applications must include a well-developed independent research plan and a statement of teaching interests and philosophy. Demonstrated success in receiving extramural funding commensurate with experience is desirable.

The University of North Dakota is committed to achieving diversity among faculty and staff and strongly encourages women and members of underrepresented groups to apply. Interested individuals must complete an electronic application at <http://www.med.und.edu/search/basicscience>, which will require submission of a letter of interest, curriculum vitae, proposed research plan, teaching statement and names of three references. Questions should be addressed to: basic.science.search@medicine.nodak.edu. Additional information about these positions and the School can be found at: <http://www.med.und.nodak.edu>. Review of applications will begin **January 3, 2011**.

The University of North Dakota is an Affirmative Action/Equal Employment Opportunity Employer. These positions are subject to a criminal background check.

GRANTS

SEEKING THE WORLD'S BEST POSTDOCS

Academic freedom
for up to five years

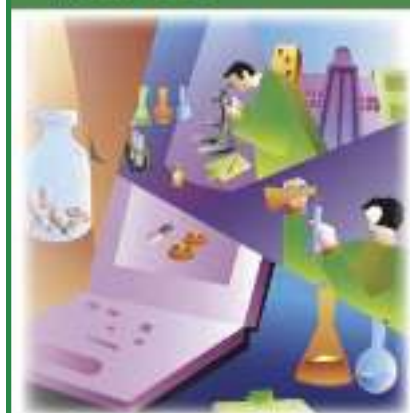
APPLY NOW!

www.society-in-science.org

society
in science
The Brando Weiss Fellowship

CAREER TRENDS

Running Your Lab



Download your free copy today at
ScienceCareers.org/booklets

Science Careers

From the journal *Science*



Brought to you by the
AAAS/Science Business Office

CHAIR, DEPARTMENT OF PEDIATRICS

UMDNJ-Robert Wood Johnson Medical School seeks candidates for the position of Chair, Department of Pediatrics. Candidates must have an MD degree or combined MD/ PhD and be Board certified and eligible for licensure in New Jersey. The desirable candidate should be of national stature, recognized for his/her accomplishments in academic Pediatrics, and possess outstanding leadership qualities. He/she must have demonstrated success in promoting excellent teaching, scholarship and research, as well as developing and implementing clinical programs.

The Department of Pediatrics supports a number of nationally and internationally renowned researchers and clinicians. The Department is part of an international center of excellence in newborn and pediatric care and child health research also consisting of the Child Health Institute of New Jersey, a biomedical research institute, and the adjacent Bristol-Myers Squibb Children's Hospital at Robert Wood Johnson University Hospital and the PSE&G Children's Specialized Hospital. Other clinical affiliations continue to develop.

As one of the nation's leading comprehensive medical schools, UMDNJ-Robert Wood Johnson Medical School is dedicated to the pursuit of excellence in education, research, health care delivery, and the promotion of community health. In cooperation with Robert Wood Johnson University Hospital, the medical school's principal affiliate, they comprise New Jersey's premier academic medical center. In addition, Robert Wood Johnson Medical School has 34 hospital affiliates and ambulatory care sites throughout the region.

As one of the eight schools of the University of Medicine and Dentistry of New Jersey with 2,500 full-time and volunteer faculty, Robert Wood Johnson Medical School encompasses 22 basic science and clinical departments and hosts centers and institutes including The Cancer Institute of New Jersey, the Center for Advanced Biotechnology and Medicine, the Environmental and Occupational Health Sciences Institute, and the Stem Cell Institute of New Jersey. The medical school maintains educational programs at the undergraduate, graduate and postgraduate levels for more than 1,500 students on its campuses in New Brunswick, Piscataway, and Camden, and provides continuing education courses for health care professionals and community education programs.

Review of applications will begin immediately and will continue until the ideal candidate is chosen. Please send nominations and/or application, including a brief statement of the attributes and qualities of the individual, visions and strategic goals for building the Department of Pediatrics and curriculum vitae to: **Alfred F. Tallia, MD, MPH, Professor and Chair, Department of Family Medicine and Community Health, Chair of Search Committee, c/o Bonnie Baloga-Altieri, PhD, RN, Director, Administration, Office of the Dean, UMDNJ-Robert Wood Johnson Medical School, 125 Paterson Street, Suite 1400, New Brunswick, New Jersey 08903, Email: balogabl@umdnj.edu. EOE.**



**ROBERT WOOD JOHNSON
MEDICAL SCHOOL**

University of Medicine & Dentistry of New Jersey



Young Group Leader Positions

Institut Pasteur announces an international call for candidates wishing to create independent young researcher groups on its Paris, France campus. The call is open to researchers specializing in cell biology, structural biology, development, epidemiology, genetics, genomics, immunology, infection, prokaryotic and eukaryotic microorganisms or viruses.

To be eligible, candidates must have defended their PhD after December 31, 2002 (derogations are possible in exceptional circumstances). Successful candidates will be appointed as head of a group of up to 6 people for a period of 5 years. The budget (up to 1,500,000€ over 5 years) includes the salary for the group leader, a three-year postdoctoral position, a technician, part-time secretarial assistance, a substantial contribution to running costs and equipment, and access to on-campus facilities including state-of-the-art technology platforms.

The Application should comprise the following (in order) in a single PDF file:

1. A brief introductory letter, including the name of the proposed group.
2. A curriculum vitae and a full publication list.
3. A description of past and present research activities (4-5 pages with 1.5 spacing).
4. The proposed research project (8-10 pages with 1.5 spacing).
5. The names of 3 scientists from whom letters of recommendation can be sought, together with the names of scientists with a potential conflict of interest from whom evaluations should not be requested.

Applications and requests for information should be addressed to the Director of Scientific Evaluation, Prof. Alain Israël at g5@pasteur.fr. The deadline for applications is January 31, 2011. Short-listed candidates will be called for interview in April 2011 and decisions will be announced by July 2011. Further information can be found on the website, <http://www.pasteur.fr>

The Norwegian University of Science and Technology (NTNU) in Trondheim represents academic eminence in technology and the natural sciences as well as in other academic disciplines ranging from the social sciences, the arts, medicine, architecture to fine art. Cross-disciplinary cooperation results in innovative breakthroughs and creative solutions with far-reaching social and economic impact.



**Faculty of Natural Sciences and Technology
Department of Biology**

Two Associate Professors in Biology (Molecular Biology)

The Department of Biology offers two vacant permanent positions.

One position in animal molecular biology (NT-41/10) and one position in plant molecular biology (NT-48/10).

For further information, please contact Head of Department, Professor Bjørn Munro Jenssen tel. +47 73 596073, e-mail: bjorn.munro.jenssen@bio.ntnu.no. More information about the department can be found on <http://www.ntnu.edu/biology>

The application with the attachments should be sent electronically through www.jobbnorge.no. **One application for each position.**

The application should be clearly marked with the reference number NT-41/10 for the position in animal molecular biology, and NT-48/10 for position in plant molecular biology. **Closing date is 15.01.2011.**

**Please see the full advertisement at www.jobbnorge.no
or visit NTNUs homepage <http://nettopp.ntnu.no>**



NTNU

Norwegian University of
Science and Technology



Nontraditional Careers: Opportunities Away From the Bench Webinar

Want to learn more about exciting and rewarding careers outside of academic/industrial research? View a roundtable discussion that looks at the various career options open to scientists across different sectors and strategies you can use to pursue a nonresearch career.

**Now Available
On Demand**
www.sciencecareers.org/webinar

Participating Experts:

Dr. Lori Conlan

*Director of Postdoc Services,
Office of Intramural Training and Education
National Institutes of Health*

Pearl Freier

*President
Cambridge BioPartners*

Dr. Marion Müller

*Director, DFG Office North America
Deutsche Forschungsgemeinschaft
(German Research Foundation)*

Richard Weibl

*Director, Center for Careers in
Science and Technology
American Association for the
Advancement of Science*

Produced by the
Science/AAAS Business Office.

Science Careers

From the journal *Science*





Executive Vice President for Health Affairs

The University of Louisville announces the search for Executive Vice President for Health Affairs (EVPHA). The successful candidate will lead the ongoing transformation of the acclaimed UofL Health Sciences Center (HSC) from a regional powerhouse to stature as a premier international academic medical center of excellence in health sciences education, research, and clinical care (www.louisville.edu/hsc).

The EVPHA will provide leadership and direction to the HSC, which includes Schools of Medicine, Dentistry, Nursing, and Public Health, the James Graham Brown Cancer Center, University Physicians' Associates (practice plans), the University Medical Center, UofL Health Care, and 17 centers and institutes. Chair of the University Hospital Board and a member of the University Medical Center board, the EVPHA has principal responsibility for HSC external affairs and fundraising. The EVPHA also serves as a member of the Office of the President, UofL's four-person senior leadership team. For detailed position description and other details: <http://www.academic-search.com/uploads/profiles/LouisvilleEVPHAProfile.pdf>.

A high research activity Research University, UofL (www.louisville.edu) is one of the nation's fastest growing institutions in biomedical research measured by NIH funding. Over the last decade, a half million square feet of new medical research space has been built. Established in 1798, the University comprises three campuses, each with new state-of-the-art research facilities: the 177-acre Belknap campus; the HSC and medical complex; and a 243-acre Shelby campus. With a diverse student body of 22,000+, UofL has \$946+ million operating budget and endowment approaching \$640 million. See video link for campus view: <http://www.youtube.com/univoflouisville#p/u/17/bBkVF9GQfU>

A vibrant metropolitan area of nearly 1 million, Louisville (www.possibilitycity.com and www.louisville.com) is one of America's leading medical communities. The local health-care industry employs more than 50,000, and there are five hospitals near the campus with 1,000+ beds. A rich cultural calendar and a remarkably low cost of living are among signature amenities.

Applications and nominations will be received until the position is filled; candidate confidentiality will be maintained until on-campus interview stage of the search. Applications should include: a letter describing relevant experiences and interest in the position; CV; names and contact information for five references. Nomination letters should include complete contact information for nominee. Submit materials electronically to: UofLEVPA@academic-search.com. For best consideration, all materials should be received before **February 25, 2011**.

Search is assisted by:

John Hicks, Senior Consultant

Academic Search, Inc.

john.hicks@academic-search.com 205-345-7221

The University of Louisville is an Affirmative Action, Equal Opportunity, Americans with Disabilities Employer, committed to diversity and, in that spirit, seeks applications from a broad variety of candidates.



ibmp
www.ibmp.fr

CNRS
Centre National de la Recherche Scientifique

Institute Director

Institut de biologie moléculaire des plantes (IBMP) –
Institute of Molecular Plant Biology

du Centre national de la recherche scientifique (CNRS) –
French National Research Center

Strasbourg/France

The Institut de biologie moléculaire des plantes (IBMP) is seeking applicants for the Scientific Director of the Institute starting January 2012.

The IBMP is the largest plant research institute of the CNRS and one of the strongest plant research centers in Europe with an international reputation for excellence in research.

The IBMP brings together world-class scientists in an intimate and highly collaborative environment, encouraging them to address the grand challenges in molecular plant biology.

Founded in 1988, the institute is world-renowned for its discoveries in genetics, epigenetics, genomics, RNAi, cell and developmental biology, biochemistry, metabolism, plant pathogen interaction, and physiology. Major lines of research are currently cell and organelle biology, metabolism, and signaling.

Staff members working in highly specialized scientific support platforms participate in this dynamic and rewarding environment and are integral to the Institute's mission.

Strasbourg, home of the European parliament and located in the heart of Western Europe, provides an excellent research environment with over 50 research institutes and one of the largest universities in France. Moreover, located between Vosges and Black Forest, Strasbourg offers very high living standards.

Applicants should have an excellent record of plant molecular biology with experience in leadership and administrative skills. The position is open to applicants of any nationality.

Interested candidates should send their CV with a letter of interest not later than 25th of March 2011 to:

Dr. Jean-Luc Eyraud (jean-luc.eyraud@ibmp.cnrs.u-strasbg.fr)

Further information can be found at: www.ibmp.fr



OREGON HEALTH & SCIENCE UNIVERSITY

Assistant Professor

Gene or Cell Therapy Faculty Position

The Department of Molecular & Medical Genetics at the Oregon Health & Science University is seeking applicants for a Gene or Cell Therapy Faculty Position. This is a full time and tenure track position at the level of Assistant Professor. Candidates who hold a PhD or MD degree are invited to apply.

The successful candidate will join a department comprised of both basic science and clinical faculty and will join an interdepartmental research initiative on gene and cell therapies. We seek outstanding candidates with the ability to direct an independent, extramurally-funded basic science or translational research program, and who will contribute to training programs in human genetics.

OHSU is known for leading excellence in health care, research and education and is the state's only health and research university. OHSU is located in Portland, Oregon, the gem of the Pacific Northwest. Affording ample cultural and outdoor experiences, Portland is considered one of the best places to live in the United States.

Apply online by visiting www.ohsujobs.com, refer to recruitment IRC32318. The application documents should include the following:

- complete curriculum vitae
- statement of research accomplishments and future research goals
- three letters of reference

Application review will begin immediately with a deadline of **January 15, 2011**. Inquiries should be addressed to **Dr. Cary Harding, Associate Professor, Chair, MMG Faculty Search Committee, c/o Dee Miller; e-mail: millerd@ohsu.edu**.

OHSU is an Equal Opportunity, Affirmative Action Institution.

POSITIONS OPEN

ASSOCIATE AND ASSISTANT PROFESSORS FACULTY ASSOCIATE Department of Biological Sciences

Seton Hall University is seeking to fill three faculty positions in the Department of Biological Sciences starting September 2011, pending final budgetary approval. One position will be at the Associate Professor level without tenure. Candidates must have a Ph.D., recent publications, five or more years experience as a research scientist and a multi-year grant that can be transferred to Seton Hall. The applicant will receive 3-4 years toward tenure and pay at the Associate Professor level.

The second position will be at the Assistant Professor level, tenure-track. Candidates must have a Ph.D. with at least two years postdoctoral training. Individuals with expertise in immunology and/or genetics are invited to apply. Research interests that include epigenetics are preferred. The successful applicant will develop a research program that involves both undergraduate and graduate students and can be externally funded. He/She will teach the equivalent of nine credits per semester, and may teach graduate courses in his/her area of expertise. The third position will be at the Faculty Associate level and is subject to budgetary approval. The Faculty Associate position has a teaching and service but not a research component. Successful applicants will hold a Ph.D in the biological sciences, preferably, and have two or more years of teaching experience in microbiology and/or anatomy & physiology. He/She will teach the equivalent of 15 credits per semester.

For consideration, please submit curriculum vitae, statements of teaching philosophy and research interests, and three letters of recommendation with cover letter to the address below. Applications are due January 17, 2011.

Seton Hall, the oldest Catholic diocesan university in the United States, is located 14 miles west of Manhattan, in South Orange, New Jersey and an enrollment of approximately 10,000 students. Candidates should be supportive of the Catholic mission of the university. *SHU is committed to programs of Equal Employment Opportunity and Affirmative Action (EEO/AA) to achieve our objectives of creating and supporting a diverse racial, ethnic, and cultural community.*

Carolyn S. Bentivegna, Ph.D.
Department of Biological Sciences
Seton Hall University
400 South Orange Avenue
South Orange, NJ 07079-2694
E-mail: carolyn.bentivegna@shu.edu
Visit our website: <http://www.shu.edu>
Equal Opportunity/Affirmative Action Employer

URBAN ECOLOGIST Florida International University

Florida International University's (FIU) Department of Biological Sciences seeks a tenure-track Urban Ecologist (open rank). The successful candidate will develop an externally funded research program; contribute to graduate and undergraduate teaching; and integrate into the Miami Urban Long-Term Research Area (ULTRA) project and the Florida Coastal Everglades LTER. Scholars interested in human interactions with all levels of ecological organization, habitats, and spatiotemporal scales will be considered. Qualifications include a Ph.D. in ecology or a related discipline, postdoctoral experience, strong publication record, demonstrated potential to obtain funding, and ability to work in interdisciplinary teams. Senior candidates should have a history of leadership in the field of Urban Ecology. Applicants should send curriculum vitae, teaching and research statements, three reprints, and contact information of four references to **Jim Heffernan**, Urban Ecologist Search chair, at **e-mail: jheffer@fiu.edu**, or to: **Department of Biological Sciences, Florida International University, Miami, FL 33199**. Review of applications will begin January 15. FIU is a member of the State University System of Florida and is an Equal Opportunity, Equal Access Affirmative Action Employer. Women, minorities, and persons with disabilities are particularly encouraged to apply.

POSITIONS OPEN

FACULTY POSITIONS in Environmental Engineering University of Notre Dame

The Department of Civil Engineering and Geological Sciences at University of Notre Dame, Notre Dame, Indiana, is accepting applications for a tenure-track position in environmental engineering. Qualified candidates at all levels, including endowed chair, will be considered, with hiring rank and tenure status commensurate with academic accomplishments. Review of applications will begin immediately, but applications will be accepted until the position is filled. Areas of interest include, but are not limited to: innovative and sustainable processes for water and wastewater management, microbiological aspects of water quality and treatment, micropollutants in water and wastewater, fate and transport of actinides in the environment, environmental effects of nanoparticles/nanoengineering, environmental sensors/sensing systems, and modeling of environmental processes and systems. The department has a unique blend of environmental engineering and environmental science faculty, and has outstanding research facilities. Current strengths include environmental microbiology, environmental geochemistry and geomicrobiology, biofilm processes, groundwater hydrology, environmental fluid dynamics, and environmental actinide chemistry and mineralogy. Information about the department and environmental engineering program can be found at **website: <http://www.nd.edu/~cegeos/>**. We seek individuals with dynamic and highly innovative research agendas that may cross traditional disciplinary boundaries. Qualifications include a Ph.D. in civil or environmental engineering or related field. Candidates are expected to exhibit a dedication to excellence in research, teaching, and professional service. The application package should include a cover letter, curriculum vitae, a statement of research and teaching interests, four references with address, e-mail, and telephone number, and up to three relevant publications. Applications should be sent electronically as a single PDF file to **Dr. Robert Nerenberg** at **e-mail: enveng@nd.edu**. *The University of Notre Dame is committed to diversity and equality in education and employment, and women and members of underrepresented minority groups are strongly encouraged to apply.*

The Department of Biological Sciences at Michigan Technological University invites applications for a tenure-track **ASSISTANT/ASSOCIATE PROFESSOR** in Plant Evolution or Systematics. Preference will be given to candidates whose research employs biochemical, molecular, and/or genetic approaches with a strong quantitative emphasis. The successful applicant will hold a Ph.D. with postdoctoral experience, and will be expected to establish a vigorous, externally funded research program. Candidates considered for Associate Professor should have an established and funded research program. All candidates must have a strong commitment to undergraduate and graduate education.

Send curriculum vitae, statement of research and teaching interests, and three letters of recommendation electronically to the search committee:

E-mail: Biosearchcommittee@mtu.edu
Biological Sciences Search Committee
Department of Biological Sciences,
1400 Townsend Drive,
Michigan Technological University,
Houghton, MI 49931-1295.

Application review will commence February 1, 2011 and continue until the position is filled.

Michigan Tech is an ADVANCE institution, one of a limited number of universities in receipt of NSF funds in support of our commitment to increase diversity and the participation and advancement of women in STEM.

Michigan Technological University is an Equal Opportunity Educational Institution/Equal Opportunity Employer.

Your career is our cause.

Get help
from the
experts.

**www.
sciencecareers.org**

- Job Postings
- Job Alerts
- Resume/CV Database
- Career Advice
- Career Forum
- Graduate Programs
- Meetings and Announcements

Science Careers

From the journal *Science*

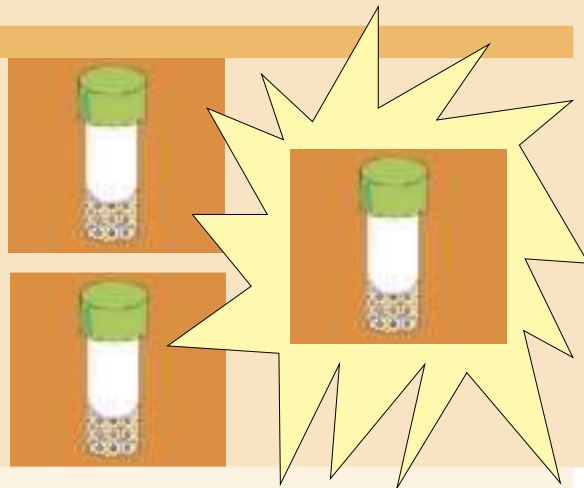
AAAS



cell sciences®

Buy two - Get three!

Buy two recombinant proteins and get an additional protein free for a limited time on any of our popular items listed below. Mention offer code RP342 when ordering. Visit www.cellsciences.com for details.



Recombinant human proteins

BD1 (47 aa)	IL6
BD2	IL8 (72 aa)
BD3	IL8 (77 aa)
BD4	IL10
BLC/BCA1/CXCL13	IL11
BMP2	IL12
BMP4	IL13
CCL14	IL15
CD40 Ligand	IL17
CNTF	IL21
CXCL1	IL31
CXCL10/IP10	IL33
CXCL11/I-TAC	LEC
CXCL2	MCP1
CXCL3/MIP2 beta	MCP2
EGF	MCP4
ENA78/CXCL5	M-CSF
Eotaxin	MEC/CCL28
Eotaxin-2	MIA-2
Exodus-2	MICA
FGF1 (aa 141)	MIF
FGF7	MIG
FGF10/KGF2	MIP-1 alpha
FLT3 Ligand	MIP-1 beta
Fractalkine	MIP-3 alpha/CCL20
GCSF	MIP-3/CCL23
GH1	MIP-4/CCL18
GM-CSF	MIP-5/CCL15
IFN alpha 2b	NAP-2/CXCL7
IFN beta 1b	Noggin
IFN gamma	NRG1
IGF1	NT4
IGFBP3	RANTES
IL1 alpha	SCF
IL1 beta	SDF-1 alpha
IL1RA	TARC/CCL17
IL2	TNF alpha
IL3	TPO
IL4	VEGF

Recombinant mouse proteins

CXCL2
CXCL16
EGF
FGF2
GM-CSF
IFN gamma
IL2
IL3
IL4
IL11
IL33
LIX/CXCL5
MCP2
Noggin
SDF-1 beta
SF20
TNF alpha
VEGF

Recombinant rat proteins

EGF
FGF2
IFN gamma
SDF-1a/CXCL12
SDF-1b/CXCL12

Other recombinant proteins

Protein A/G
Staphylokinase
Streptokinase

Offer good for any combination of three items on the list above. Products are for research use only. Not for human use. Not for use in diagnostic or therapeutic procedures.

www.cellsciences.com

INTRODUCING AAAS **MemberCentral**



The exclusive new website for the AAAS member community.

AAAS MemberCentral is a new website focused on helping you — the scientists, engineers, educators, students, policymakers, and concerned citizens who make up the AAAS community — connect like never before.

On MemberCentral you can contribute to discussion groups or blogs, participate in a webinar, or share photos of your field research. You can exchange ideas, learn about your fellow members, and gain fresh insights into issues that matter to you the most. MemberCentral is also an easy access point for a wide variety of other AAAS membership benefits, like discounts on cars and books, travel opportunities, and more.

Experience MemberCentral for yourself. Visit MemberCentral.aaas.org today and log in using your *Science* online username and password.



MemberCentral.aaas.org

GE Healthcare
Life Sciences

Inspired to Accelerate scale-up

Inspired by your need for fast and predictable scale-up, we introduce ÄKTA™ avant 150, the latest development in our ÄKTA avant series.

Optimize your process with ÄKTA avant 25 and then scale-up seamlessly to ÄKTA avant 150 by automatically converting your methods using UNICORN™ 6.1 software.

ÄKTA avant is designed to fully exploit the advantages of our modern BioProcess™ media like MabSelect™ and Capto™.

Dedicated column formats ensure seamless scalability from process development to manufacturing – from prepacked HiScreen™ columns to HiScale™ to AxiChrom™ with Intelligent Packing.

Want to know more? Register today to receive a copy of the ÄKTA avant 150 Data File.
www.gelifesciences.com/pr-avant

GE, Imagination at work, and GE monogram are trademarks of General Electric Company. ÄKTA, AxiChrom, BioProcess, Capto, HiScale, HiScreen, MabSelect, and UNICORN are trademarks of GE Healthcare companies.
© 2010 General Electric Company – All rights reserved.
First published Sept. 2010
GE Healthcare Bio-Sciences AB, Björksgatan 30, 751 84 Uppsala, Sweden
GE09-10



imagination at work

Free up your time



Automated sample and assay technologies by QIAGEN

Automated solutions from sample to result:

- The widest choice of sample processing protocols
- Low-, medium-, and high-throughput automation
- Leading solutions for molecular diagnostics
- Plug-and-play automated sample preparation
- Quantitative, real-time PCR detection
- Automated analysis of DNA fragments and RNA
- High-resolution sequence-based DNA detection and quantification
- HPV testing

AUTOBIO11051WV

Making improvements in life possible — www.qiagen.com



Sample & Assay Technologies



"How do we know
this lead molecule
is novel?"

“SciFinder—
of course.”

Need to assess the novelty of substances?

SciFinder is the answer.

It includes CAS REGISTRYSM, the most comprehensive substance information available, integrated with relevant journal articles and patents.

Give your research team the highest quality and most timely scientific information resource.

Make SciFinder an essential part of your research process.

For more information about SciFinder, visit www.cas.org or e-mail help@cas.org.

an essential
✓
SciFinder®—Part of the process.™



CAS is a division of the American Chemical Society

www.cas.org

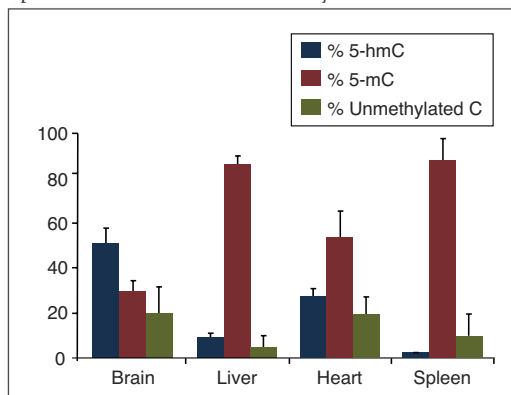


MASTERING CHANGE

Breakthrough in 5-hmC quantitation for epigenetics

Interested in simplifying the study of DNA methylation, particularly 5-hydroxymethylcytosine? Try the EpiMark™ 5-hmC and 5-mC Analysis Kit, a robust enzymatic method for the locus-specific detection of methylated (5-mC) and hydroxymethylated (5-hmC) cytosine. As the first commercially available PCR-based kit to reproducibly identify and quantitate the presence of 5-hmC, this simple 3-step protocol will expand your potential for epigenetics research and biomarker discovery.

Identify and quantitate methylation states with the EpiMark™ 5-hmC and 5-mC Analysis Kit



Analysis of the different methylation states in Balb/C mouse tissue samples shows a variation in the amount of 5-hmC present at locus 12.

Advantages:

- Reproducible quantitation of 5-hmC and 5-mC
- Easy-to-use protocols
- Compatible with existing techniques (PCR)
- Amenable to high throughput

Visit neb.com/epigenetics to learn more, and explore the complete listing of EpiMark validated products from NEB.



CLONING & MAPPING

DNA AMPLIFICATION
& PCR

RNA ANALYSIS

PROTEIN EXPRESSION
& ANALYSIS

GENE EXPRESSION
& CELLULAR ANALYSIS

www.neb.com

Luminex



Why limit yourself?

Experience more with MAGPIX[®] and perform up to 50 tests in a single reaction volume.

This groundbreaking new instrument gives you more data with greater efficiency – all at a price you won't believe.

USE MAGPIX TO:

- Transition from single to multiplex analysis as your research grows
- Analyze both proteins and nucleic acids on a single platform
- Benefit from extensive vendor support and a broad menu of assay kits
- Reduces benchtop clutter with a smaller, compact instrument
- Accelerate your research with a solution that costs up to 4X less than comparable ELISA assays*



The question isn't whether you can afford MAGPIX.
It's whether your research can afford to continue *without it*.

Find out how affordable multiplexing can be by visiting Luminexcorp.com/MAGPIX.

MAGPIX[®]

Multiply the Power of Your Potential.



*Based on a single assay kit. Luminex MAGPIX assays are typically 4X less expensive than comparable ELISA assays.



Because science never stands still.

Introducing the **FREE Mobile App** from **Science**. See what it can do for you.

Now you can access *Science* from anywhere. Our new app lets you:

- Read summaries and abstracts from *Science*, *Science Translational Medicine*, and *Science Signaling*.
- E-mail yourself links to full text.
- Stay updated with *ScienceNOW*, our free daily news service.
- Search job openings and tap other resources at *Science Careers*.
- Access the *Science* weekly podcast and other multimedia.
- Store content for reading without wi-fi access.

Get it for your iPhone or iPod Touch. Get it for FREE. Get it today.

To download the free app visit the iTunes App Store or log on to
content.aaas.org/mobile



'Tis the season of giving (and getting)

Give *Science* with full AAAS benefits.
Get our special gift rate and our
new shirt!

\$99 Professional gift rate
(reg. \$146)

\$50 Postdoc/Student gift rate
(reg. \$99/\$75)



Make the holidays happier for a promising young researcher, family member, friend, or student. *Science* is the gift that lasts all year—and includes all the benefits of membership in AAAS.

Get our new “Explain Your Research” shirt—FREE! With its playful graphics, it makes just the right fashion statement at holiday gatherings, and other social events all year long.

You’ll enjoy something else as well: the satisfaction of helping to support AAAS and our international, public policy, and educational programs—the ones that advance science and serve society. Happy holidays, indeed!

**Order now: visit promo.aaas.org/holiday
or call 866-434-2227.**



**A \$22.50 value—
yours FREE when you
give *Science*!**

AAAS + U = Δ

Detail on back

Please order at least two weeks before the holiday you’re celebrating, so we have time to send your gift recipient a letter announcing your gift. Non-U.S. recipients may receive *Science* Digital edition at the special gift rate. Check online for print edition rates. \$74 allocated to *Science* for Professional memberships, \$50 for Postdoc/Student memberships. Please allow 4 weeks for receipt of first issue. Prices valid through 3/1/11.





Learn how current events are impacting your work.

ScienceInsider, the new policy blog from the journal ***Science***, is your source for breaking news and instant analysis from the nexus of politics and science.

Produced by an international team of science journalists, *ScienceInsider* offers hard-hitting coverage on a range of issues including climate change, bioterrorism, research funding, and more.

Before research happens at the bench, science policy is formulated in the halls of government. Make sure you understand how current events are impacting your work. Read *ScienceInsider* today.

www.ScienceInsider.org

*Science***Insider**

Breaking news and analysis from the world of science policy



NEW PRODUCTS



PROTEIN G BIOSENSOR

The Dip and Read Protein G biosensor can be used for rapid detection and quantification of numerous types of mammalian immunoglobulins (IgGs) from solution. Because it runs on the company's label-free Octet instrumentation platform, the new biosensor enables such measurements with unprecedented speed, ease-of-use, and cost-efficiency. The Protein G biosensor can be regenerated and reused for increased workflow flexibility and cost-efficiency. The biosensor comes with recombinant Protein G pre-immobilized on the surface and is ready-to-use on Octet instruments. All Octet biosensors are designed to simplify kinetic characterization and quantitation by eliminating throughput and time-to-result limitations of traditional surface plasmon resonance-based assays and enzyme-linked immunosorbent assays. The Octet biosensors are disposable, configured in a standard microplate format, and are coated with a proprietary biocompatible matrix that is uniform, non-denaturing, and has minimal nonspecific binding.

ForteBio, Inc.

For info: 650-322-1360 | www.fortebio.com

YEAST PROTEIN EXTRACTION

The YPX Yeast Protein Extraction Kit is unlike other yeast protein extraction products since it does not use mechanical disruption to break open the tough cell walls of yeast. Using the YPX kit is much faster than carrying out conventional yeast lysis procedures used for protein extraction, and results in a higher total protein yield. The protocol is simple and economical, and offers substantial usability and cost benefits to researchers who wish to carry out experiments on many yeast cultures simultaneously. The YPX Yeast Protein Extraction Kit allows extraction of the entire yeast proteome with nearly complete efficiency, and is uniquely compatible with quantitative yeast proteomics workflows, which would otherwise be complicated by day-to-day and lab-to-lab variations in protein extraction efficiencies. The economical kit contains sufficient material to extract proteins from 150 grams of spun-down, wet cell pellet yeast culture harvests. Starting material can be frozen or freshly harvested.

Protein Discovery

For info: 865-521-7400 | www.proteindiscovery.com/extraction/

ChIP-VALIDATED ANTIBODIES

With Thermo Scientific Pierce Chromatin Immunoprecipitation (ChIP)-validated antibodies, researchers can obtain results more quickly by avoiding the testing of multiple antibodies to determine their suitability in ChIP assays. These antibodies are a perfect complement to the recently launched Thermo Scientific Pierce Agarose ChIP Kit. Thirty ChIP-validated antibodies are available to common targets, including numerous histone and transcription factor proteins.

Thermo Fisher Scientific

For info: 815-968-0747 | www.thermoscientific.com/pierce

GEL DOCUMENTATION SYSTEM

Gel Doc EZ is a compact gel documentation system that provides publication quality images and analysis in seconds—with just the push of a button—for researchers who perform DNA, RNA, and protein electrophoresis, as well as Western blotting. The Gel Doc EZ im-

ager requires no training for first-time users and provides automated push-button functionality. This eliminates the need for researchers to manually manipulate filters, lenses, or lighting, thereby minimizing the potential for human error. In addition, the new system may be expanded to meet a variety of imaging applications. The imager allows the use of multiple application-specific trays, including a ultra-violet tray for imaging fluorescent stains such as ethidium bromide, a white tray for imaging colorimetric stains such as Coomassie blue, a blue tray for imaging SYBR Green-stained DNA, and a stain-free tray for imaging proteins separated by electrophoresis on Bio-Rad's Criterion TGX Stain-Free precast gels. The Gel Doc EZ imager is the only gel documentation system capable of imaging stain-free gels, an advanced gel technology that condenses traditionally long staining protocols down to a five-minute activation and imaging process.

Bio-Rad

For info: 800-424-6723 | www.bio-rad.com

TOXICITY DETECTION

The MaxDiscovery Aspartate Transaminase (AST) and Lactate Dehydrogenase (LDH) Color Endpoint Assays use a proprietary, innovative technology for visible detection of in vivo toxicity using only 5 μ L of serum from rodents or other mammals. These assays are powerful tools for the detection of drug-induced toxicity to the liver and heart can be used for preclinical testing in the drug discovery process. Both assays employ simplified endpoint analysis to offer high sensitivity, low detection limits, and the ability to use a visible plate reader. The MaxDiscovery AST kit permits quantitative visible determination of AST in a convenient format. Unlike traditional LDH tests, the MaxDiscovery LDH Color Endpoint Assay kit measures the direct chemical conversion of LDH and does not rely on coupled enzymatic amplification for high precision, quantitative measurements. Each kit contains ready-to-use product standards for the development of standard curves and accurate calibration of the assay.

Bioo Scientific

For info: 888-208-2246 | www.biooscientific.com

Electronically submit your new product description or product literature information! Go to www.sciencemag.org/products/newproducts.dtl for more information.

Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and governmental organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by *Science* or AAAS of any products or materials mentioned is not implied. Additional information may be obtained from the manufacturer or supplier.

Call for Papers

Science Translational Medicine

Integrating Medicine and Science

Chief Scientific Adviser

Elias A. Zerhouni, M.D.

*Senior Fellow, Global Health Program,
Bill & Melinda Gates Foundation*

*Former Director,
National Institutes of Health*

Senior Scientific Adviser

Elazer Edelman

*Thomas D. and Virginia W. Cabot Professor
of Health Sciences and Technology
Massachusetts Institute of Technology*

Editor

Katrina L. Kelner, Ph.D.

Managing Editor

Orla M. Smith, Ph.D.

Senior Editor

Kelly LaMarco, Ph.D.

Science Translational Medicine, from AAAS, the publisher of *Science*, focuses on the conversion of basic biomedical research into practical applications, thus bridging the research-to-application gap, linking basic scientists and researchers.

Submit your manuscripts for review in the following areas of translational medicine:

- Cardiovascular Disease
- Neuroscience/Neurology/
Psychiatry
- Infectious Diseases
- Cancer
- Health Policy
- Bioengineering
- Chemical Genomics/
Drug Discovery
- Applied Physical
Sciences
- Drug Delivery
- Gene Therapy/
Regenerative Medicine
- Cell Culture, Animal,
and Human Studies
- Other Interdisciplinary
Approaches to Medicine

Submit your research at
www.submit2scitranslmed.org

Subscribing to **Science Translational Medicine** ensures that you and your lab have the latest translational medicine resources. For more information, visit ScienceTranslationalMedicine.org



For more information see
ScienceTranslationalMedicine.org or
contact scitranslmededitors@aaas.org



ScienceTranslationalMedicine.org

Proteins

Antibodies

ELISAs

Assay Services

MultiAnalyte Profiling

Activity Assays

Stem Cells

ELISpot Kits

Flow Cytometry

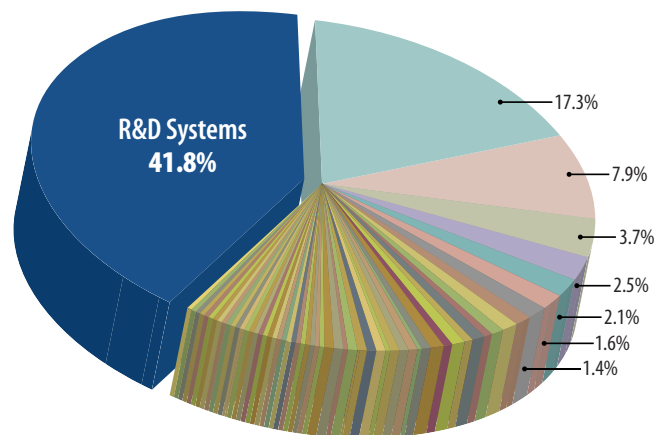
Cell Selection

R&D Systems Quantikine® ELISAs

The Most Referenced Immunoassays

A direct measure of product quality is the frequency of citations in the scientific literature. R&D Systems has more than 20 years of experience designing, testing, and optimizing the most cited ELISA kits in the world. Find out why scientists trust R&D Systems ELISAs more than any other brand.

R&D Systems is the Most Referenced ELISA Manufacturer



Approximately 42% of Referenced Immunoassays are Developed and Manufactured by R&D Systems. A survey of 860 manuscripts from 44 journals was conducted to compare the number of citations specifying the use of R&D Systems ELISAs to the number citing ELISAs from other commercial sources. A total of 433 ELISA citations referencing immunoassays from 66 different vendors were identified in the survey.

NEW Quantikine ELISA Kits

- α 1-Acid Glycoprotein
- Angiopoietin-like 3
- Cathepsin V
- Clusterin
- Dkk-1
- EGF R/ErbB1
- EG-VEGF/PK1
- Fetuin A
- FGF-21
- Galectin-3
- Gas 6
- GDF-15
- IL-17A/F Heterodimer
- IL-19
- Lipocalin-2/NGAL
- MBL
- Proprotein Convertase 9/PCSK9
- Periostin/OSF-2
- Progranulin
- ST2/IL-1 R4
- Thrombomodulin/CD141
- Tie-1
- TIM-1/KIM-1

For more information visit our website at www.RnDSystems.com/go/ELISA

For research use only. Not for use in diagnostic procedures.

R&D Systems, Inc. www.RnDSystems.com

R&D Systems Europe, Ltd. www.RnDSystems.co.uk

R&D Systems China Co., Ltd. www.RnDSystemsChina.com.cn

R&D
SYSTEMS®